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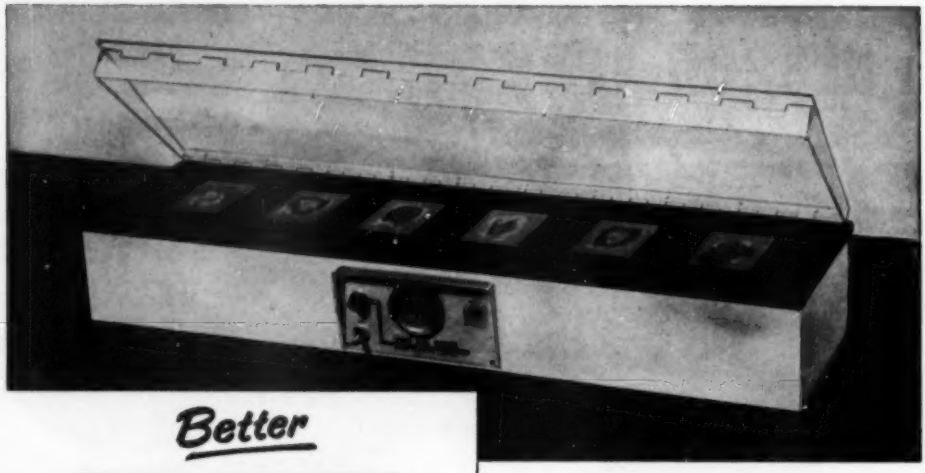

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STUDIES IN FIBROSIS OF THE LIVER INDUCED BY CARBON TETRACHLORIDE

I. Relation Between Hepatocellular Injury and New Formation of Fibrous Tissue

K. ATERMAN, M.D., M.R.C.P., D.C.H.
BIRMINGHAM, ENGLAND

OF THE many hepatotoxic agents which, if given over prolonged periods of time, lead to the formation of fibrous tissue in the liver, carbon tetrachloride is the one most commonly used.[1] The part it plays in the production of fibrosis of the liver is not well understood; its action in this respect can, however, be influenced by the administration of cortisone.* In the present paper the effect of cortisone on the amount of fibrous tissue in the liver has been assessed quantitatively by measuring its collagen content. Some histological observations are reported because of the light they throw on the relation of the lesions of the hepatic cells to the newly formed fibrous tissue.

DEVELOPMENT OF FIBROSIS

A survey of the literature shows a lack of agreement about the distribution of the fibrous tissue in the liver of animals subjected to prolonged treatment with carbon tetrachloride (CCl_4). Whereas Bollman[4] and Lacquet[5] state that the fibrous tissue was formed in the central zone of the liver lobule, Cameron and Karunaratne[6] maintain that only a collapse fibrosis "can be seen there which is entirely subsidiary to the proliferation of the connective tissue around the portal tracts." Since then many investigators have accepted the periportal origin of the fibrous tissue trabeculae,[1] but Ashburn, Endicott, Daft, and Lillie[7] showed conclusively by injection methods the relation of the fibrous bands to the central hepatic veins. Himsworth,[8] who later apparently modified his view,[9] was not prepared to abandon the notion of the periportal origin of the fibrous tissue, although he realized the difficulty inherent in this concept. For with two,† possibly three,‡ exceptions, practically every investigator in the field has described the hepatocellular lesions produced by CCl_4 as being characteristically localized to the central zone of the lobules.§ This, therefore, raises the fundamental question of the relation of the newly formed fibrous tissue to the degenerative cellular lesions. Is there any reason to believe that fibrosis of the liver caused by CCl_4 is an exception to the general rule that fibrous tissue forms where parenchymatous cells have been damaged or destroyed? In order to reconcile centrally located cellular lesions with peripheral fibrous tissue proliferation, one would have either to postulate with Cameron and

Department of Anatomy, Medical School, University of Birmingham.

* References 2 and 3.

† References 10 and 11.

‡ Maegraith, B. G., in discussion on Morrione.[18]

§ References 5, 6, 7, 8, 12, 13, 14, 15, 16, 17, and 18. Hartroft, W. S., in discussion on Morrione.[18] Bennett, G. A., in discussion on Morrione.[18]

Karunaratne [6] a lymphatic, or with Himsworth [8] an "as yet unexplained" spread of cellular disintegration products from one part of the lobule to another, or consider with Gillman and Gillman [19] that, ". . . while alterations in the cytological appearance of the hepatic epithelium can frequently be correlated with hepatic fibrosis, there does not seem to be any relation between these parenchymal changes and hepatic fibrosis." To many observers Moon's [20] concept of hepatic fibrosis being "like other chronic inflammations the result of parenchymatous injury followed by repair . . .," which has only recently been questioned again by Morrione,‖ would appeal more than either of these views, if such a sequence of events could actually be demonstrated. This relation between hepatocellular injury and new formation of fibrous tissue is the subject of the present paper.

METHODS

Two groups of animals have been studied. Group I comprises 42 animals taken from the experiment to be described in detail in the following paper. They were female albino rats purchased from the Agricultural Research Council (Compton Field Station), weighing at the start of the experiment from 85 to 171 gm., average 121 gm. Throughout the experiment they were fed the stock rat cake diet, but one batch of 10 rats received a daily supplement of 10 mg. of calcium pantothenate per 10 gm. of diet. Carbon tetrachloride (British Drug Houses) was given subcutaneously twice weekly in a dose of 0.1 ml. for 9 injections and 0.12 ml. for another 20 injections. Ten rats, as well as the batch receiving the pantothenate, were killed at the end of this period (14 weeks). The dose of CCl_4 was now for a time increased to 0.15 ml., and 10 animals were killed after the 63d injection (31 weeks). The administration of CCl_4 was then discontinued, and the surviving rats were allowed to recover for five weeks to see if the fibrosis had reached the irreversible phase. Seven animals died or had to be killed at various stages in the course of the experiment after having received from 21 to 54 injections of CCl_4.

Group II is taken from an experiment described elsewhere [21] and includes eight male albino rats of an inbred strain used in this laboratory, weighing from 150 to 200 gm., average 177 gm., at the start of the experiment. They were given 0.2 ml. of CCl_4 subcutaneously in intervals of two to three days, so that they received 21 injections over a period of seven and a half weeks.

The animals were killed by bleeding; pieces of the liver were fixed in 5% formaldehyde (Formol)-saline or formaldehyde-Zenker solution, and 7-μ sections were stained with hematoxylin and eosin, Mallory's azocarmine stain, and Foot's silver impregnation stain for reticulin.

RESULTS

The different survival times and the well-known variations in susceptibility to CCl_4, not only in different strains but also in individual rats, made it possible to observe the evolution of the fibrous tissue bands from the stage of the localized, purely hepatocellular damage to the stage of the irreversible fibrosis. The early changes in the liver cells have been repeatedly described ¶ as consisting of changes in the staining properties of the cytoplasm, nuclear pyknosis and complete dissolution, cellular vacuolation, and "hydropic degeneration." Attention will be paid in the present paper only to the most conspicuous sign of cellular damage, the "hydropic degeneration," or the "balloon-like" [6] change of the liver cells. The cell in this condition swells, sometimes quite considerably; the cytoplasm stains much lighter than is normal, becomes granular, and assumes a ground-glass appearance (Fig. 10); the nucleus becomes small and rather dense. According to Cameron and Karunaratne,[6] such cells can appear quite empty, except for a few darkly staining

‖ Morrione,[18] p. 181.

¶ References 1, 5, 6, 7, 8, 13, 14, 15, 16, and 21.

shreds of cytoplasm converging toward a pyknotic nucleus. The appearance is quite characteristic, as is the arrangement of these cells, for they can always be found either immediately beneath the capsule (Fig. 2) or surrounding the central hepatic veins. From there they extend in rows, presumably along the branches of these vessels, to map out a pseudolobular pattern (Fig. 1). The cells surrounding the portal tracts remain intact. If, however, the damage becomes more extensive, or if

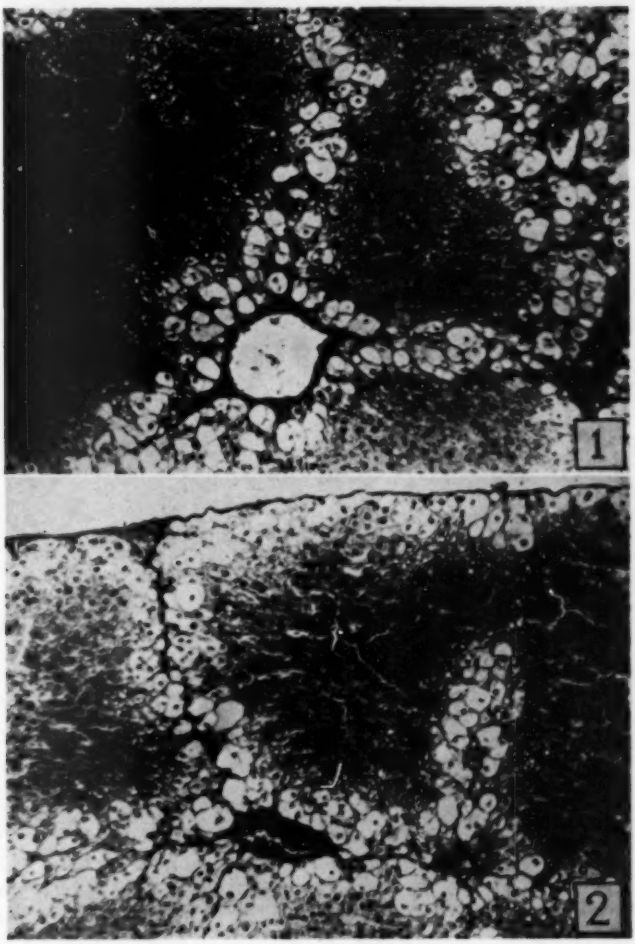

Fig. 1.—Figures 1 to 12 show the relation of the newly formed fibrous tissue to areas of cellular damage in the liver of rats treated with carbon tetrachloride. Male rat given 21 injections of 0.2 ml. CCl_4 over period of seven and one-half weeks. Marked hydropic change of the liver cells around the central vein, but no fibrous tissue can be seen. Five per cent formaldehyde-saline solution fixation, 7 μ section; Mallory's azocarmine stain; × 100.

Fig. 2.—Same animal as in Figure 1. Subcapsular vacuolation, with beginning fibrous tissue formation. Five per cent formaldehyde-saline solution fixation; 7 μ section; Mallory's azocarmine stain; × 100.

a hepatic vein can be found in the section in close proximity to a portal tract, a real or apparent extension of the cellular lesions towards the periportal region can be observed.

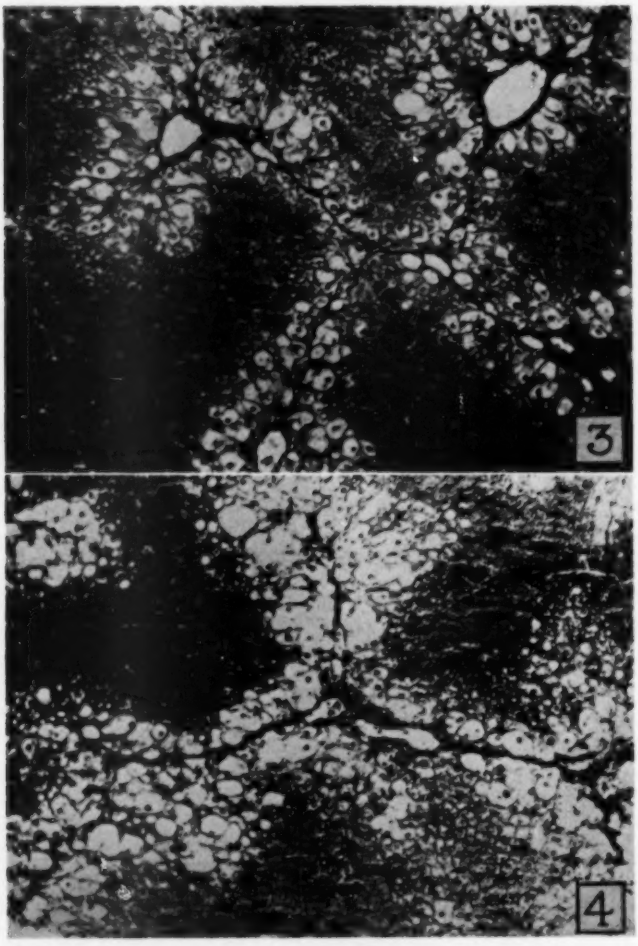

Fig. 3.—Male rat given 21 injections of 0.2 ml. CCl_4 over period of seven and one-half weeks. Definite but fine fibrous tissue strands connecting hepatic veins. Note the arrangement of the damaged liver cells. Five per cent formaldehyde-saline solution fixation; 7 μ section; Mallory's azocarmine stain; × 100.

Fig. 4.—Male rat given 21 injections of 0.2 ml. CCl_4 over period of seven and one-half weeks. The fibrous tissue formation is becoming pronounced. Five per cent formaldehyde-saline solution fixation; 7 μ section; Mallory's azocarmine stain; × 100.

These changes become apparent a few hours after a single injection of CCl_4.[5] Fibrous tissue formation, however, only takes place after an interval of varying length, depending presumably on the animals' susceptibility, during which the

administration of CCl_4 must be continued. Connective tissue stains reveal in this interval, the stage of hepatocellular damage, only a thickening of the basement membranes of the sinusoids (Fig. 8), but definite fibers are still absent (Fig. 1). When they first are observed, they appear as delicate strands which are always surrounded by hydropic cells (Figs. 2, 3, 9, and 10). They are therefore best seen

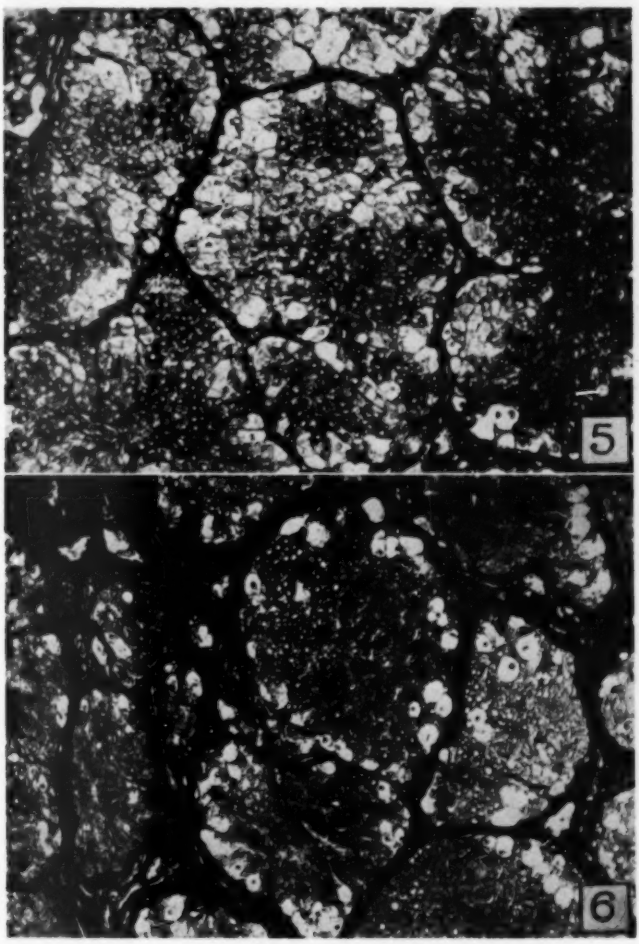

Fig. 5.—Female rat given 29 injections of 0.1 to 0.12 ml. CCl_4 over a period of 14 weeks. The architecture of the liver is obliterated; the fibrosis is marked. The fibrous tissue strands are surrounded by hydropic cells, the number of which is beginning to decrease. Formaldehyde-Zenker solution fixation; 7 μ section; Mallory's azocarmine stain; $\times$ 100.

Fig. 6.—Female rat given 62 injections of 0.1 to 0.15 ml. CCl_4 over a period of 31 weeks. This animal had been treated with cortisone, without effect. Advanced fibrosis, with marked decrease in the number of hydropic cells. Note their striking localization to the close vicinity of the fibrous tissue bands. Formaldehyde-Zenker solution fixation; 7 μ section; Mallory's azocarmine stain; $\times$ 100.

either extending from the capsule into the parenchyma (Fig. 2) or in the vicinity of branches of the hepatic veins, but they cannot be found in the periportal regions. The emergence of these fibers appears to be a gradual process, since in the early

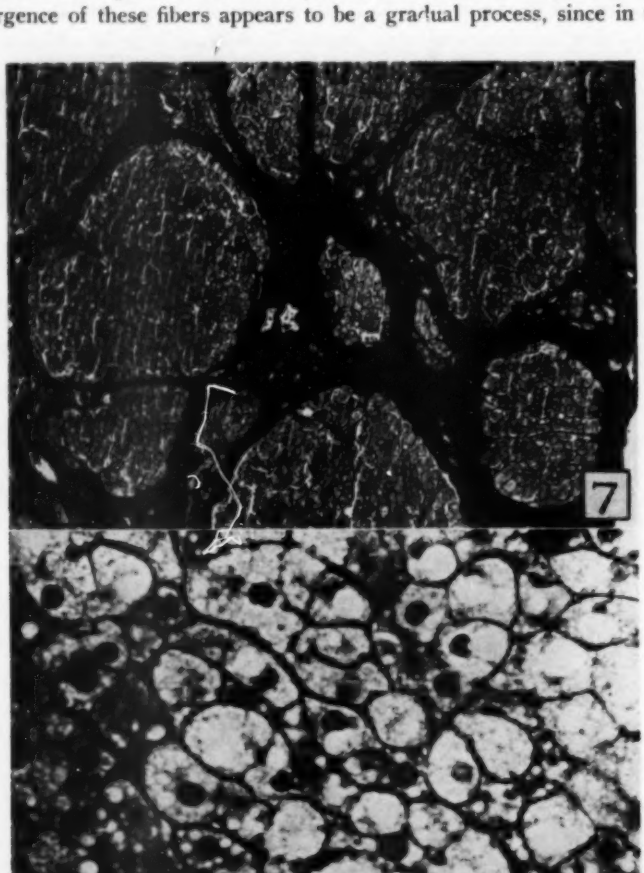

Fig. 7.—Female rat given 62 injections of 0.1 to 0.15 ml. CCl_4 over a period of 31 weeks. Advanced fibrosis, with complete absence of hydropic cell changes. Formaldehyde-Zenker solution fixation; 7 μ section; Mallory's azocarmine stain; × 100.

Fig. 8.—Male rat given 21 injections of 0.2 ml. CCl_4 over period of seven and one-half weeks. Marked hydropic cell changes, with thickening of the basement membranes of the sinusoids. Five per cent formaldehyde-saline solution fixation; 7 μ section; Mallory's azocarmine stain; × 450.

stages they are not seen throughout the section, despite the presence of a number of foci of hepatocellular damage. As the lesion progresses, however, they become more numerous, increase in thickness (Figs. 10, 11, and 12), and show a distinct

tendency to connect branches of the hepatic veins (Fig. 3). Owing to their characteristic localization to areas of cell damage, they tend to reproduce the pseudolobular pattern mentioned earlier in the paper. It is easy to see how an extension of this process will lead to the disorganization of the lobular pattern and the vascular

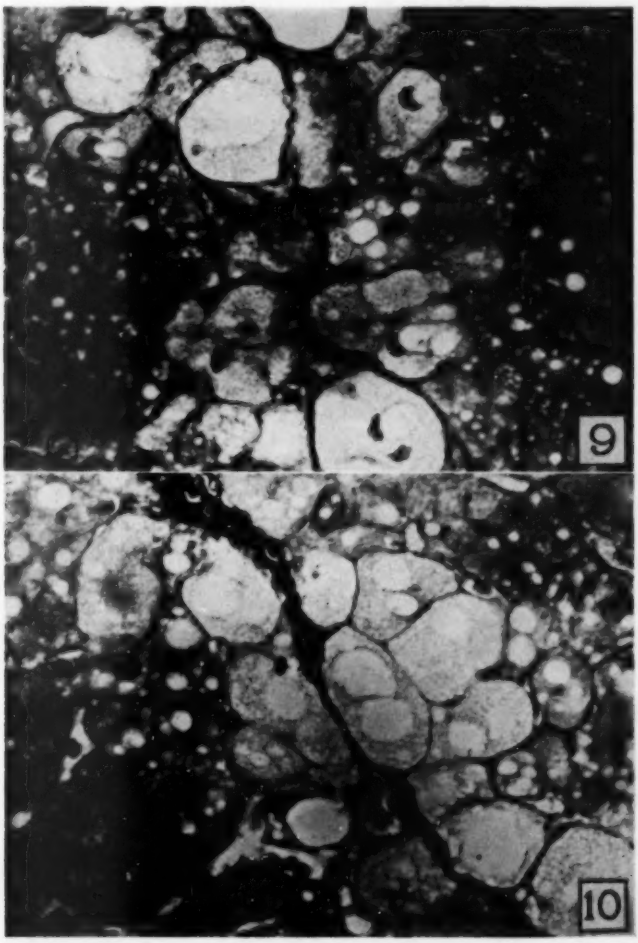

Fig. 9.—Same animal as in Figure 2. The new formation of connective tissue strands is just beginning. Five per cent formaldehyde-saline solution fixation; 7 μ section; Mallory's azocarmine stain; × 450.

Fig. 10.—Same animal as in Figure 4. The fibrous tissue strands are becoming definite. The intimate relation of hepatic cell damage to the new formation of fibrous tissue is easily seen. Five per cent formaldehyde-saline solution fixation; 7 μ section; Mallory's azocarmine stain; × 450.

architecture of the liver (Figs. 4, 5, 6, and 7). The periportal areas are still unchanged, but with the continuation of the injections the cellular changes, with the subsequent fibrous tissue proliferation, gradually extend to include the portal vessels.

Once the fibrosis is established, an increase in the amount of connective tissue deposited appears to take place partly by the continued new formation of fibers but mainly by a continuous growth in thickness of the trabeculae already formed (Figs. 4, 5, 6, 7, 10, 11, and 12). Hydropic cells are still present and show a marked

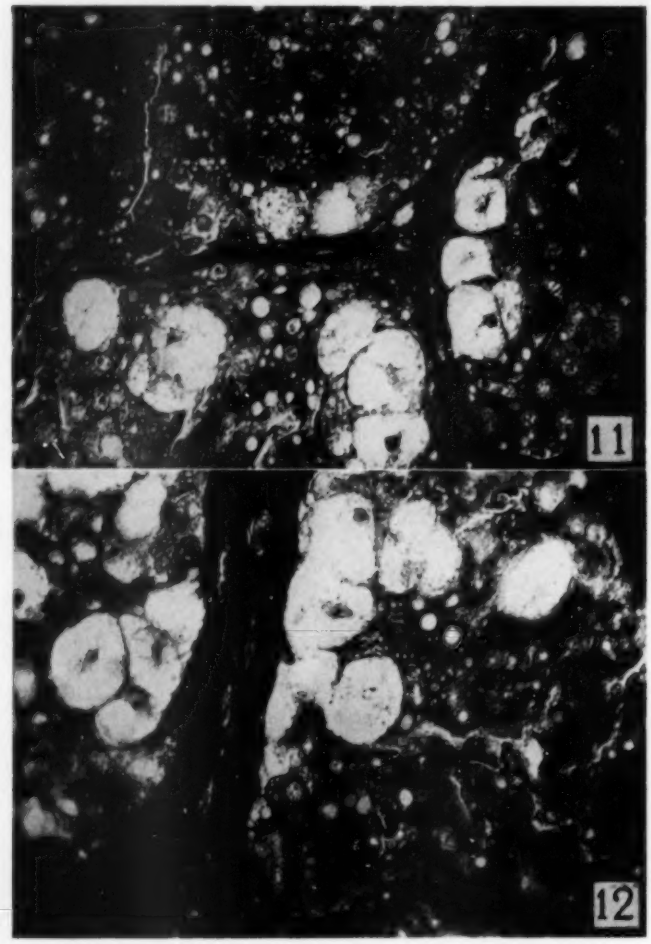

Fig. 11.—Same animal as in Figure 6. Advanced liver fibrosis, with definite peritrabecular localization of the hydropic cells. Five per cent formaldehyde-saline solution fixation; 7 μ section; Mallory's azocarmine stain; × 450.

Fig. 12.—Same animal as in Figure 11. The decrease in the number of damaged cells is clearly brought out.

tendency to occur in close vicinity to the fibrous bands (Figs. 5, 6, 10, 11, and 12), as if the growth of these trabeculae depended on the continued presence of injured liver cells. This peculiar association has also been noted by Edwards and Dalton [22] in the livers of mice given carbon tetrachloride for one month or more. Gradually,

however, a definite decrease in the number of hydropic cells is becoming apparent (Fig. 6), so that in the advanced fibrosis they may finally be altogether missing (Fig. 7). The gradual transition from the early stage of hepatocellular damage, with many hydropic cells but no or only little fibrous tissue, to the late stages of the irreversible fibrosis, with few or no hydropic cells, was such as to give the impression that an increase in the fibrosis protected the remaining liver cells from being damaged by CCl_4.

COMMENT

The association of cellular changes with the new formation of fibrous tissue bands was so conspicuous and so constant that there could be little doubt about their causal connection. It is therefore neither necessary to postulate the transport of cellular disintegration products from one zone of the liver lobule to another nor

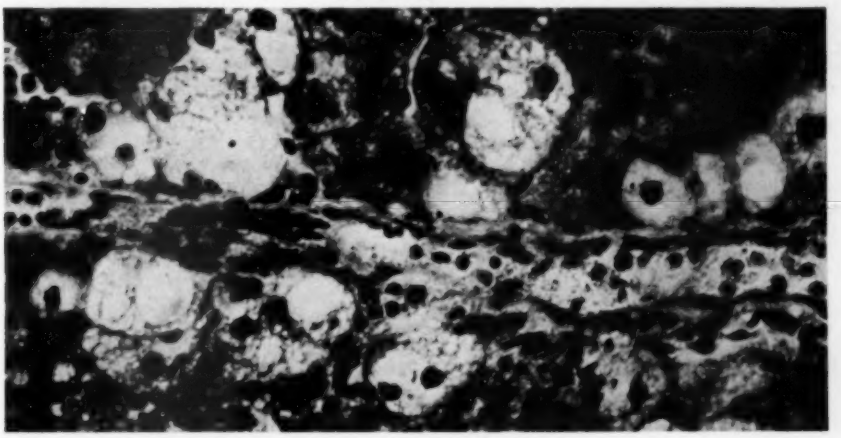

Fig. 13.—Female rat given 29 injections of 0.1 to 0.12 ml. CCl_4 over a period of 14 weeks. Vascular channel in a fibrous tissue band. Formaldehyde-Zenker solution fixation; 7 μ section; hematoxylin and eosin; $\times$ 450.

justifiable, at least as far as poisoning with CCl_4 is concerned, to consider the development of the fibrosis as a process independent of the preceding liver damage.

The occurrence of connective tissue fibers in the centrolobular areas of cell damage has been described by Cameron and Karunaratne [6] but was considered by them to represent the "fibrosis of collapse," a process subsidiary to the proliferation of fibrous tissue in the portal areas. This interpretation is not readily applicable to the present findings. The constantly observed marked swelling of the damaged liver cells; the gradual increase in the number and thickness of the fibers; their constant association with hydropic cells, even in the established fibrosis, and the relatively late involvement of the portal tract speak against the assumption of a fibrosis of collapse as distinct from the new formation of connective tissue.

It is not difficult to understand why the proliferation of the fibrous tissue should start in the areas of liver cell damage. It is less easy to explain why in the established fibrosis the damaged cells should continue to show such a remarkable tendency to congregate in the neighborhood of the connective tissue trabeculae. The

reason may perhaps be found in the rearrangement of the vascular architecture which takes place in fibrosis of the liver. Moschcowitz [23] has drawn attention to the vascularization of the fibrous tissue bands in this condition and stressed the occurrence of vascular communications between branches of the hepatic veins. Some observations made in the present series confirm this view; the tendency of the fibrous tissue bands to connect branches of the hepatic veins has already been commented upon, and the presence of blood vessels in the fibrous tissue trabeculae could frequently be noted (Fig. 13). It is well known that in acute poisoning with CCl_4 the degenerative cellular changes are limited to a greater or lesser extent to the cells surrounding the hepatic veins, although the reason for this preferential localization is not quite clear. In view of the presence of vascular channels, presumably derived from the hepatic veins, in the connective tissue trabeculae it is probable that this tendency to preferential localization of the hydropic cell changes to the neighborhood of these bands is determined by the continued operation of the factor which influences their localization in the acute stage.

This line of reasoning could be pursued still further to account for the gradual reduction in the number of hydropic cells with increasing fibrosis. Moschcowitz [23] has also pointed out that in the later stages of fibrosis direct communications between branches of the portal and the hepatic veins, "minor Eck's fistulae," as he called them, are being formed. This gradual rearrangement may affect the course of chronic CCl_4 poisoning in two ways. Either less and less CCl_4 can now come into contact with liver cells which would bind it, because it is now continually short-circuited into the general circulation, where it may affect other organs to a greater extent than before and thus perhaps contribute to death in the later stages, or, alternatively, cellular changes will be seen only as long as there are "vulnerable" cells available. A stage may finally be reached when, owing to the continuous replacement fibrosis, only just as many hepatic cells are left as can be fully protected by the factor(s) present in the portal blood. On the other hand, the gradual reduction in the occurrence of hydropic cells may well be the result of the animals' acquiring "tolerance" toward CCl_4. This question can only be answered when more is known about the factors which determine the animals' susceptibility toward CCl_4.

SUMMARY

The development of fibrosis of the liver has been studied in rats receiving small doses of CCl_4 for prolonged periods. The association of the newly formed connective tissue fibers with preceding hepatocellular damage was so constant as to suggest their causal connection, thus supporting Moon's concept of hepatic fibrosis. The suggestion is advanced that the same vascular factors which determine the localization of the cellular lesions in acute damage by CCl_4 continue to be operative throughout the stage of chronic poisoning.

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STUDIES IN FIBROSIS OF THE LIVER INDUCED BY CARBON TETRACHLORIDE

II. A Quantitative Study of the Effect of Cortisone on Fibrosis of the Liver in Rats

K. ATERMAN, M.D., M.R.C.P., D.C.H.
BIRMINGHAM, ENGLAND

IN A HISTOLOGICAL study of the changes brought about by cortisone in early fibrosis of the liver induced by carbon tetrachloride,[1] it had been noticed that an occasional rat failed to show that cortisone had inhibited the development of fibrous tissue. This observation raised the question whether the effect of cortisone was limited only to a certain phase in the development of the liver fibrosis, corresponding to the "reversible stage" of Cameron and Karunaratne,[2] and suggested a study of its effects on the more advanced degree of liver damage. Since histological examination alone was not considered adequate for an assessment of the amount of fibrous tissue present in the late stages of liver fibrosis, it was decided to determine quantitatively the collagen content of the liver.

METHODS

Eighty-four female rats purchased from the Agricultural Research Council (Compton Field Station) and weighing at the start of the experiment from 85 to 171 gm. (average 121 gm.) were used. They were divided into seven groups of 10 animals each, the experimental animals, and one group of 14 animals, which were kept as normal weight controls. All rats were fed the standard rat cake diet *; one group of experimental animals, however, was, in addition, given a daily supplement of 10 mg. of calcium pantothenate per 10 gm. of the diet. This group will be described in detail in the following paper. All experimental animals received biweekly subcutaneous injections of redistilled carbon tetrachloride (CCl_4) according to the schedule shown in Table 1. After the 26th injection of CCl_4 cortisone acetate (Cortone [Merck]) was given daily in single intramuscular injections to one group of rats for 10 days, so that each rat received a total of 71.25 mg. The injections of CCl_4 were continued during this period, at the end of which, on the day after the 29th injection of CCl_4, the animals were killed simultaneously with a group of rats treated with CCl_4 only. The 14 untreated weight controls and the group of rats fed the supplements of calcium pantothenate were killed at the same time. These animals represent Experiment 1, designed to study the stage of early fibrosis of the liver (14 weeks).

In the remaining rats the biweekly dose of CCl_4 was now increased for a time (Table 1), and after the 60th injection cortisone was given to 11 animals in the same dose as in Experiment 1. Again the injections of CCl_4 were continued during the period of cortisone administration. The day after the 63d injection of CCl_4 those rats that had survived were killed with a control group of 10 rats treated with CCl_4 only. These animals represented Experiment 2, illustrating the stage of advanced fibrosis (31 weeks).

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* Composition of the rat cake diet was: ground oats 17.7%, wheat offal 17.7%, ground wheat 17.7%, full cream-dried milk 14.0%, meat and bone meal 8.8%, maize meal 8.8%, ground barley 8.8%, white fish meal 4.5%, dried yeast 1.2%, cod liver oil 0.4%, salt 0.4%.

In five animals the injections of CCl_4 were now discontinued, and a recovery period of five weeks was allowed to pass to see if the fibrosis had reached the irreversible stage.

In all the groups food, but not water, was withdrawn for 15 hours before termination of the experiment; the animals were weighed before death which was brought about by bleeding under light anesthesia with ether. The liver was rapidly dissected and weighed, and representative samples, weighed to the nearest milligram, were taken for the determination of the collagen content, frozen in liquid nitrogen, and stored at a temperature of — 30 C. till used. The amount of collagen present was estimated by the method of Neuman and Logan,[3] based on the determination of the hydroxyproline content, but the correction for tyrosine was omitted. The water content was calculated on weighed samples of the remainder of the liver, which were stored in propanol; the latter was repeatedly evaporated in vacuum flasks till the weight of the sample became constant. The difference between the value thus obtained and the wet weight of the sample gave the amount of water present in the liver. The samples were then transferred, with repeated chloroform washings of the flasks, to weighed thimbles and were extracted with a hot mixture of two-thirds of chloroform and one-third of methanol till their weight was again constant. The loss of weight thus found represented the lipid content of the sample.

TABLE 1.—*Treatment Accorded to Animals*

No. of Animals	Treatment	Injections					
		1st-10th	11th-25th	26th-29th	30th-52d	53d-59th	60th-63d
Experiment 1 (early fibrosis) *							
10	CCl_4 only	0.1 ml.	0.12 ml.	0.12 ml.	.....	.....	.....
10	CCl_4 plus cortisone	0.1 ml.	0.12 ml.	0.12 ml. plus 71.25 mg. cortisone (7.125 mg./day)	.....	.....	.....
Experiment 2 (late fibrosis) †							
10	CCl_4 only	0.1 ml.	0.12 ml.	0.12 ml.	0.15 ml.	0.1 ml.	0.1 ml.
11	CCl_4 plus cortisone	0.1 ml.	0.12 ml.	0.12 ml.	0.15 ml.	0.1 ml.	0.1 ml. plus 71.25 mg. cortisone (7.125 mg./day)

* Injections over a period of 14 weeks.
† Injections over a period of 31 weeks.

Specimens for histological examination were fixed in 5% formaldehyde (Formol)-saline or formaldehyde-Zenker's solution. Seven-micron sections were stained with hematoxylin and eosin, Mallory's azocarmine stain for connective tissue, and Foot's silver impregnation method for reticulin. The degree of fibrosis was assessed by marking the sections 0 to 5+, according to the amount and the appearance of the fibrous tissue present. In general the presence of thick fibrous strands was considered to indicate a more advanced stage of fibrosis than an equal amount of fibrous tissue present in the form of more numerous thin fibers. Figures 1 to 6 are representative examples of the system of marking used.

RESULTS

A. The Effect of Carbon Tetrachloride and of Cortisone on the Weight and on the Composition of the Liver

EXPERIMENT 1.—*Early Fibrosis.*—After 14 weeks of treatment with CCl_4 no significant difference could be found in the appearance or in the weight of the animals given injections compared with the normal controls (average body weight 193.4 gm. compared with 194.3 gm.). The liver, however, was significantly ($P<0.02$) heavier (8.89 ± 0.23 gm. compared with 5.69 ± 0.13 gm.) and appeared fatty. The surface was finely nodular in some, whereas it was smooth in other ani-

mals. The liver margin was frequently irregular, and the consistency of the parenchyma on cutting felt distinctly tough and rubbery. In one animal free fluid was found in the peritoneal cavity. The content of fat was significantly ($P < 0.05$) raised (7.66

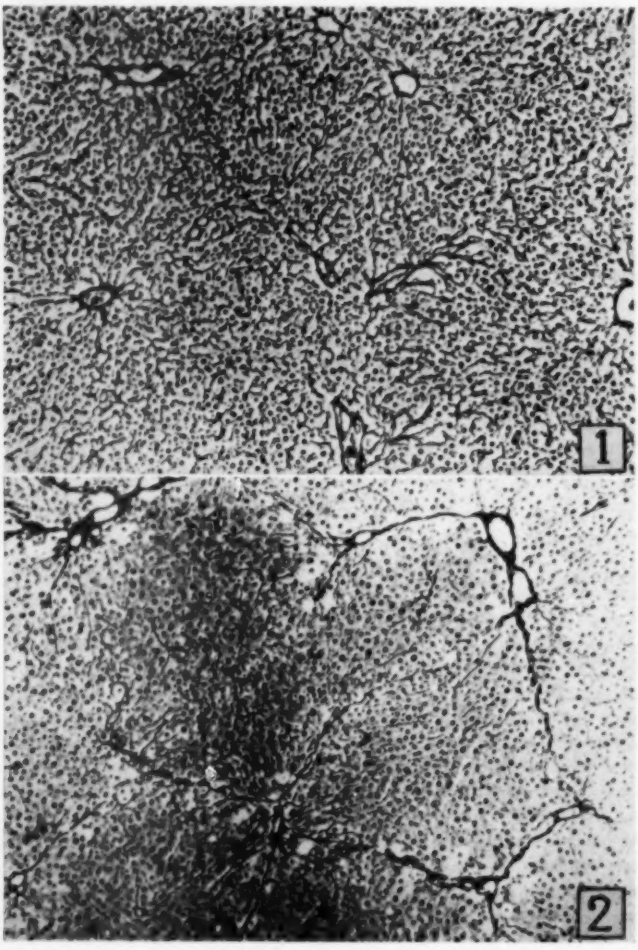

Fig. 1.—Degree of fibrosis, 0. Figures 1 to 6 show representative sections of the liver illustrating the system of marking used in assessing the degree of fibrosis. Five per cent formaldehyde-saline solution fixation; 7 μ section; Foot's silver impregnation stain for reticulin; × 100.

Fig. 2.—Degree of fibrosis, 1+. Five per cent formaldehyde-saline solution fixation; 7 μ section; Foot's silver impregnation stain for reticulin; × 100.

± 0.82% compared with 5.64 ± 0.43%), but the content of water was not significantly different (72.19 ± 1.08%).

Cortisone, given to animals treated with CCl_4, produced a rapid loss of weight from an average of 191.3 to 145.3 gm. during the 10-day period of its administration.

One animal died before the course was completed. By comparison with the group of animals treated with CCl_4 alone the average liver weight was significantly decreased (6.32 ± 0.30 gm.) and was not significantly different from the normal values. All the livers in this group appeared very fatty; the surface was smooth, and the margin was rounded and regular; on cutting they felt soft. The fat content

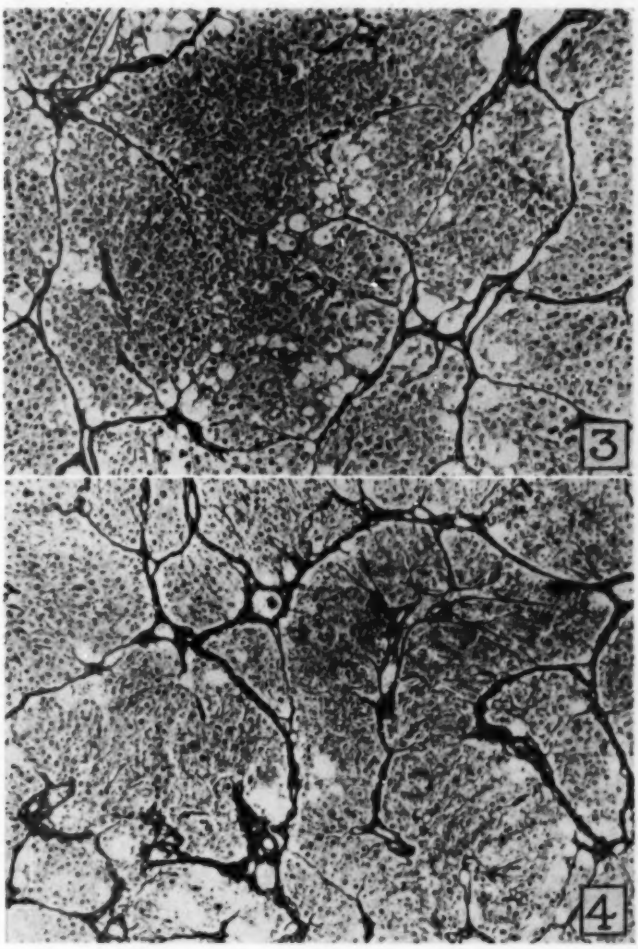

Fig. 3.—Degree of fibrosis, 2+. Five per cent formaldehyde-saline solution fixation; 7 μ section; Foot's silver impregnation stain for reticulin; × 100.

Fig. 4.—Degree of fibrosis, 3+. Five per cent formaldehyde-saline solution fixation; 7 μ section; Foot's silver impregnation stain for reticulin; × 100.

was the highest of the whole experiment ($10.8 \pm 0.76\%$); the content of water was $68.65 \pm 0.94\%$. The spleen appeared considerably smaller in all of the animals given cortisone.

EXPERIMENT 2.—*Late Fibrosis.*—Despite the prolonged administration of CCl_4, the animals continued to grow, so that their average weight at autopsy was 213.0 gm. The weight of the liver was not significantly different from that of animals with early fibrosis (8.35 ± 0.49 gm.), but its average fat content had returned to the normal value ($5.22 \pm 0.56\%$). No significant changes were found in the water

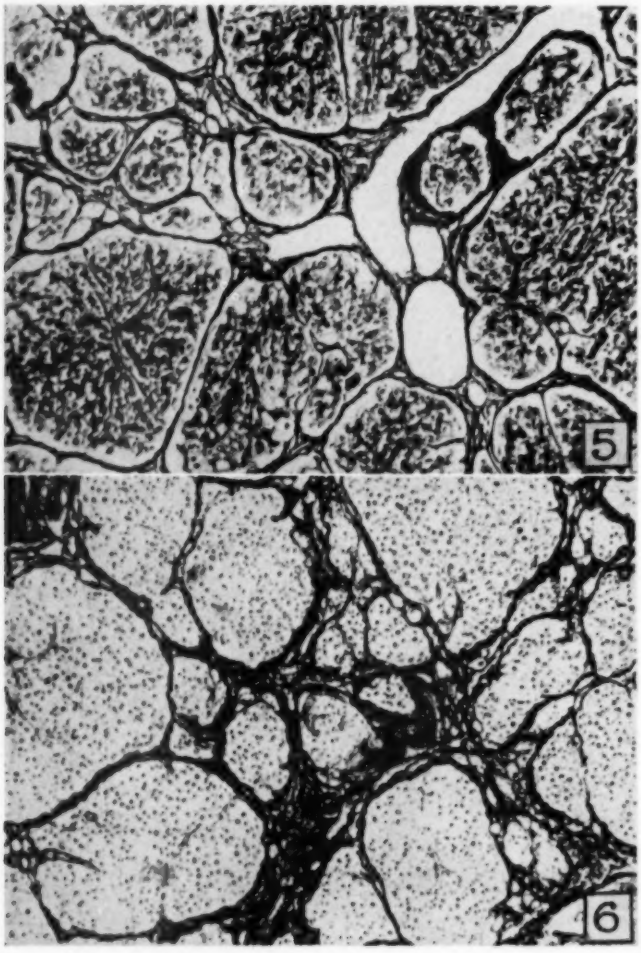

Fig. 5.—Degree of fibrosis, 4+. Five per cent formaldehyde-saline solution fixation; 7 μ section; Foot's silver impregnation stain for reticulin; × 100.

Fig. 6.—Degree of fibrosis, 5+. Five per cent formaldehyde-saline solution fixation; 7 μ section; Foot's silver impregnation stain for reticulin; × 100.

content. The liver was bound by adhesions to the diaphragm and to the omentum; its surface was coarsely nodular; its margin was irregular, and it felt very tough on cutting. In two animals there was free fluid in the peritoneal cavity.

Cortisone again led to a decrease in the average body weight from 207 to 174 gm. but had little effect on the average weight of the liver (7.61 ± 0.38 gm.). Of 11 animals in this group, 6 died or had to be killed before the 10-day course of treatment with cortisone was completed. No obvious cause of death could be found. In three animals free fluid was present in the peritoneal cavity, in one also in the

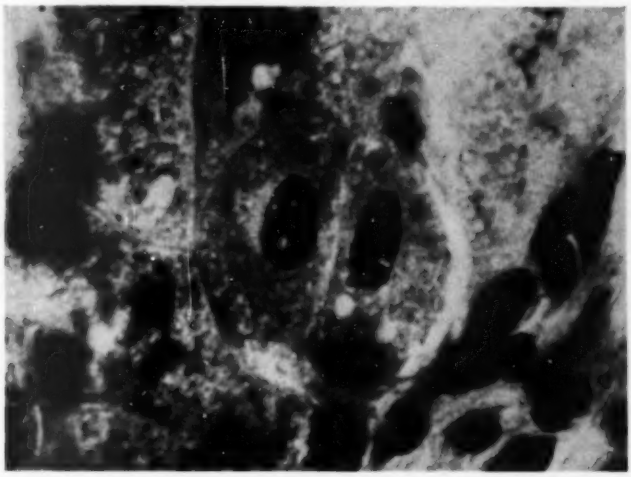

Fig. 7.—Postmitotic phase in a hepatic cell seen in the markedly fibrosed liver of a rat treated with CCl_4 for 31 weeks. Formaldehyde-Zenker solution fixation; 7 μ section; hematoxylin and eosin; × 900.

TABLE 2.—*The Effect of Cortisone on Fibrosis of the Liver Induced by Carbon Tetrachloride*

	No. of Rats											
	Mallory's Azocarmine Stain						Reticulin (Foot) Stain					
Treatment	0	+	++	+++	++++	+++++	0	+	++	+++	++++	+++++
Experiment 1 (early fibrosis)												
CCl_4	—	2	1	4	3	—	—	—	3	4	3	—
CCl_4 plus cortisone...	2	5	2	1	—	—	1	3	4	2	—	—
Experiment 2 (late fibrosis)												
CCl_4	—	—	—	1	2	7	—	—	—	1	3	6
CCl_4 plus cortisone...	—	—	1	4	—	5	—	—	—	3	2	5

pleural cavity. The liver in most animals differed little in appearance from that of animals treated with CCl_4 only, but in three rats it had a smooth surface with a regular rounded margin and cut very easily. Of interest was the fact that the administration of cortisone in this group did not produce the significant increase in the fat content seen in the liver of rats with early fibrosis, the average now being $6.13 \pm 1.04\%$; whereas the water content was $72.85 \pm 1.55\%$. The size of the spleen had decreased from an average of 1.08 gm. in animals treated with CCl_4 alone to 0.63 gm.

TABLE 3.—*Correlation Between Collagen Content and Degree of Fibrosis of the Liver*

No. of Animal	Mg. Collagen per Gm. Liver (Wet Weight)	Degree of Fibrosis		Mg. Collagen per Gm. Liver (Dry Weight)
		Mallory's Azocarmine Stain	Reticulin (Foot) Stain	
1	1.468	....	....	5.994
7	1.548	....	....	6.271
81	1.586	....	....	.....
2	1.656	....	....	6.570
82	1.722	....	....	.....
3	1.746	....	....	7.064
33	1.841	....	....	7.262
6	1.906	....	....	8.408
40	1.965	....	....	8.108
83	1.978	....	....	.....
22	1.985	+	++	9.014
39	2.000	+	+	9.087
16	2.085	....	+	9.513
10	2.112	....	....	8.499
36	2.164	....	....	8.798
11	2.192	+	++	10.063
84	2.346	....	....	.....
8	2.395	....	....	10.270
30	2.496	++	++	12.364
60	2.659	+	++	12.088
42	2.697	++	++	12.338
55	2.797	+	++	12.721
21	2.836	+	+	12.907
65	2.950	++	++	13.409
26	3.214	++	+++	15.489
79	3.216	+++	+++	15.105
60	3.335	+++	+++	14.910
70	3.614	+++++	++++	17.170
51	3.786	+++	+++	17.211
69	3.911	+++	+++	19.025
63	4.171	+	++	18.963
41	4.490	+++++	+++++	20.411
19	4.699	+++	+++	21.012
24	4.759	+++	+++	21.612
52	4.984	+++	+++	22.657
58	5.128	+++	+++	23.312
80	5.296	+++++	+++++	24.074
76	5.531	++++	++++	25.142
72	5.549	++++	++++	25.226
77	6.148	++++	++++	30.740
5	6.178	+++	++++	26.040
57	6.462	++++	++++	29.375
73	6.694	++++	++++	33.163
66	6.701	+++	++++	31.247
39	6.782	++++	+++	30.830
17	6.955	++++	+++++	33.505
13	7.104	+++	++++	32.294
48	7.615	+++++	++++	34.617
45	9.815	+++++	+++++	46.579
56	9.944	++++	++++	45.201
47	10.235	+++++	+++++	46.523
18	10.848	+++++	+++++	49.311
75	10.931	+++++	+++++	54.238
28	11.349	++++	++++	56.021
9	12.068	+++++	+++++	54.718
64	13.241	+++++	+++++	60.187
31	13.267	+++++	+++++	62.009
34	14.406	+++++	+++++	70.296

B. The Effect of Carbon Tetrachloride and of Cortisone on the Collagen Content of the Liver

Histological examination at the end of 14 weeks (Experiment 1) showed that CCl_4 had produced a fibrosis which ranged from 0 to 4+. Most of the animals belonged to categories 3 to 4+ (Table 2). Despite the continued administration of CCl_4 cortisone produced a reduction in the degree of fibrosis (Table 2). The histological features of this effect have been described elsewhere [1]; they are, briefly, a disappearance of some of the abnormal fibers and a marked attenuation of the remaining connective tissue elements. The thinning of the connective tissue strands seen in Mallory's azocarmine stain, as well as in the silver stain, was considered to be characteristic of the effect of cortisone. Most animals of this group were graded as belonging to categories 1 to 2+.

At the end of 31 weeks (Experiment 2) the severity of the fibrosis had increased, most of the animals belonging to category 5+ (Table 2). Cortisone given now had little effect on the degree of fibrosis, although a characteristic attenuation of the fibrous trabeculae could still be seen in two animals.

Table 4.—*The Correlation Between the Histological Appearance of the Liver and Its Collagen Content*

Silver Stain for Reticulin	Collagen, Mg. per Gm. Wet Liver	Total Collagen, Mg.
0*	1.881 ± 0.091	10.691 ± 0.537
+	2.308 ± 0.265	14.124 ± 0.797
++	2.779 ± 0.265	16.855 ± 1.625
+++	4.511 ± 0.369	26.522 ± 4.560
++++	7.121 ± 0.838	66.984 ± 8.827
+++++	11.050 ± 1.070	83.799 ± 8.637

* Normal.

The histological findings are compared with the chemical determination of the collagen content in Table 3. Since the conclusions reached in the present paper were essentially the same, whether the collagen content was calculated on the basis of the wet or the dry fat-free weight of the liver, reference will only be made to the values for the wet weight. It can be seen that the agreement between the histological and the chemical assessment, though not exact, is on the whole good; with increasing severity of the fibrosis, as judged by the histological appearance, the collagen content increases too (Table 4). In only one animal (Rat 63) was there a serious discrepancy; no explanation can be offered for this unusual result. In general, the silver impregnation method for reticulum appeared to reflect more closely than Mallory's azocarmine stain the amount of fibrous tissue present, as shown by the chemical estimation. This finding recalls Morrione's † conclusion concerning the greater sensitivity of the reticulum stain as compared with the trichrome stain in assessing the amount of connective tissue.

Detailed comparisons of the histological and chemical determinations for the various treatment groups are shown in Tables 5 and 6. The fibrosis caused by the administration of CCl_4 for 14 weeks (Experiment 1) raised the average total collagen of the liver from 10.69 ± 0.54 mg. in the normal controls to 45.66 ± 8.17 mg. or from 1.89 ± 0.09 mg. per gram of liver tissue to 4.98 ± 0.80 mg. ($P < 0.01$).

† References 4 and 5.

TABLE 5.—*The Effect of Cortisone on the Collagen Content of the Liver in Early Fibrosis (Fourteen Weeks, Experiment 1)*

Normal Controls			CCl_4					CCl_4 plus Cortisone				
No. of Animal	Collagen, Mg. per Gm. Wet Liver	Total Collagen, Mg.	No. of Animal	Collagen, Mg. per Gm. Wet Liver	Total Collagen, Mg.	Mallory's Azocarmine Stain	Silver Stain	No. of Animal	Collagen, Mg. per Gm. Wet Liver	Total Collagen, Mg.	Mallory's Azocarmine Stain	Silver Stain
1	1.468	8.208	22	1.986	15.606	+	++	59	2.001	13.116	+	+
7	1.548	8.857	50	2.659	22.272	+	++	16	2.086	13.558	0	+
2	1.659	8.974	65	2.950	24.841	++	++	11	2.192	14.183	+	++
3	1.746	9.005	60	3.335	27.961	+++	+++	42	2.098	16.606	++	++
33	1.842	10.756	69	3.911	34.007	+++	+++	55	2.797	15.507	+	++
6	1.906	12.296	19	4.700	39.478	+++	+++	21	2.837	15.699	+	+
40	1.996	12.483	57	6.463	60.426	++++	++++	26	3.214	19.735	++	+++
10	2.113	11.410	38	6.783	69.523	++++	+++	51	3.786	31.909	+++	+++
36	2.164	11.233	13	7.105	68.384	+++	++++	63	4.172	22.830	+	++
8	2.395	13.080	56	9.944	98.974	++++	++++	..	.....	.....	..	..
Mean	1.881 ± 0.091	10.691 ± 0.537		4.984 ± 0.795	45.061 ± 8.167				2.865 ± 0.250	18.139 ± 2.012		

TABLE 6.—*The Effect of Cortisone on the Collagen Content of the Liver in Late Fibrosis (Thirty-One Weeks, Experiment 2)*

CCl_4					CCl_4 plus Cortisone				
No. of Animal	Collagen, Mg. per Gm. Wet Liver	Total Collagen, Mg.	Mallory's Azocarmine Stain	Silver Stain	No. of Animal	Collagen, Mg. per Gm. Wet Liver	Total Collagen, Mg.	Mallory's Azocarmine Stain	Silver Stain
70	3.614	30.649	+++++	++++	41	4.491	29.369	+++++	+++++
52	4.985	33.049	+++	+++	24	4.760	38.125	+++	+++
17	6.955	78.188	++++	+++++	58	5.129	34.773	+++	+++
48	7.616	60.478	+++++	++++	5	6.179	55.097	+++	++++
45	9.816	90.991	+++++	+++++	60	6.701	55.640	+++	++++
47	10.235	62.087	+++++	+++++	18	10.848	90.191	+++++	+++++
28	11.350	111.229	++++	++++	34	14.467	93.310	+++++	+++++
9	12.038	86.253	+++++	+++++		.....	.....		
64	13.241	103.612	+++++	+++++		.....	.....		
21	13.367	120.190	+++++	+++++		.....	.....		
Mean	9.312 ± 10.71	77.672 ± 9.774				7.511 ± 1.416	56.617 ± 9.806		

The large variation in the amounts of collagen present is considered to be an indication of the varying susceptibility of individual animals to poisoning with CCl_4, to which White,[6] Brunschwig, Johnson, and Nichols,[7] and Daniel, Prichard, and Reynell [8] have drawn attention. Cortisone, given to a similar group, significantly ($P < 0.02$) reduced the average total collagen content to 18.14 ± 2.01 mg. or to 2.87 ± 0.25 mg. per gram of liver tissue but did not succeed in restoring it to the normal level. Whereas the liver weight in this group decreased by about 28.8% of the average liver weight found in the group of animals treated with CCl_4 only, the corresponding reduction in the amount of collagen per gram of liver tissue was approximately 42.7%. Another interesting feature of the effect of cortisone was the greatly reduced variance noted in this group. The meaning of this lessened variability is not clear, but it is tentatively interpreted as an expression of the tendency to revert to the norm.

After 31 weeks of treatment with CCl_4 (Experiment 2) the amount of collagen present in the liver per gram of tissue had significantly ($P < 0.05$) increased above that present in the early fibrosis to 9.31 ± 1.07 mg. (Table 6). The average total collagen content amounted to 77.67 ± 9.77 mg. Cortisone effected a slight reduction in the content of collagen (56.62 ± 9.80 mg. or 7.51 ± 1.41 mg. per gram of liver) which, however, was not considered to be significant.

C. Recovery

Autopsy of five animals five weeks after the last (62d) injection of CCl_4 showed a fibrosis of the liver ranging from 1 to 5+. It is therefore probable that most animals of Experiment 2 had reached the stage of irreversible fibrosis of the liver described by Cameron and Karunaratne.[2]

COMMENT

The lower fat content of the liver with advanced fibrosis after 31 weeks of treatment with CCl_4, as compared with that in the milder fibrosis after only 14 weeks, recalls a similar observation by Chaikoff, Eichorn, Connor, and Entenman [9] and by Himsworth and Glynn [10] in fibrosis of the liver of dietary origin. In the preceding paper it has been pointed out that *pari passu* with the increase in the degree of fibrosis there can be seen a decrease in the number of hydropic cells, which are generally considered to be an index of hepatocellular damage. It is possible that the same factors which are responsible for this curious phenomenon are also the cause of the changes in the fat content, an increase of which frequently accompanies liver cell damage. Whether the failure of the markedly fibrosed liver to respond to the administration of cortisone with the increase in the percentage of fat seen in the early stages of liver fibrosis can also be attributed to the same cause must at present remain unanswered.

Another point of interest is the significant difference in the number of animals which survived the 10 days of treatment with cortisone. Whereas only 1 animal out of 10 with early fibrosis died prematurely, 6 out of 11 rats with advanced fibrosis failed to survive the course. This is in agreement with the observation [11] that, despite the favorable effect of cortisone on the connective tissue, the function of the liver, as seen by the sulfobromophthalein sodium (Bromsulphalein) retention test, is adversely affected by cortisone in chronic poisoning with CCl_4.

It has generally been accepted, mainly on the basis of studies on wound healing, that cortisone exerts its effect on the connective tissue by inhibiting or depressing its new formation.‡ Granulation tissue that has already been deposited is stated not to be affected by cortisone.[24] It is questionable whether the findings presented here can be explained solely as the result of the decreased new formation of fibrous tissue during the period of cortisone administration. If the amount of new collagen deposited daily per gram of liver is calculated for the period of the early fibrosis, the advanced stage, and the whole experiment, the average figures of 31.66 γ, 36.37 γ, and 34.2 γ, respectively, are arrived at. Despite the continued administration of CCl_4, however, the average daily decrease under the influence of cortisone is, in the early stages of fibrosis, at least five and a half times as great. Although according to Morrione § the rate of fibrous tissue formation in experimental fibrosis of the liver is not uniform throughout the experiment but becomes more rapid as the lesion progresses, the decrease in the total amount of collagen, the striking decrease in the variability of the individual values, and the characteristic attenuation of the connective tissue elements suggest an active removal and not merely a decrease in the formation of the abnormal fibrous tissue under the influence of cortisone. This interpretation is supported by the observation of Cameron and Karunaratne,[2] Morrione,|| and Steinberg and Martin [26] that fibrosis of the liver is not an irreversible process; by the statement of Morrione [5] that such a reversal takes place by the dissolution and thinning of the connective tissue fibers, and by the finding of Baker [27]; Castor and Baker [28]; Winter, Silver, and Stoerk,[29] and Cavallero and Braccini [30] that a thinning and loss of substances of the connective tissue elements of the normal dermis can be produced by the application of adrenocortical steroids such as cortisone.

The failure of cortisone to affect the histological appearance and the collagen content of the liver in advanced fibrosis is of interest. It is, of course, possible, though not probable in view of the high mortality, that a still higher dosage than the one used in the present experiments would have produced a significant difference in the amount of collagen in the liver. Alternatively the explanation of this failure may well be found in changes taking place in the connective tissue itself; these may either be related to changes in the degree of polymerization of the connective tissue components, as suggested by the hypothesis of Gersh and Catchpole,[31] or may be connected with the transition from "procollagen" to "collagen."[32] The fibrous tissue has become "mature" and can now no longer be broken down under the influence of cortisone.

The findings presented here bear a certain similarity to the observation of Cameron and Karunaratne [2] that the reversible fibrosis of the liver gradually passes into an irreversible stage, and raise the question whether this change is determined by an alteration in the state of the adrenal function. It is well known that the formation of fibrous tissue bands in the liver occurs only after a latent interval, varying presumably with the animals' susceptibility, during which the administration of CCl_4 has to be continued. Conceivably the first injections of CCl_4 produce in the animal a state of "stress," to which the adrenals respond with an increase in the secretion of their cortical steroids. Owing to the raised level of the steroids, the

‡ References 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, and 23.

§ References 5 and 25.

|| References 5 and 25.

proliferation of fibrous tissue elements is prevented, and histological examination will therefore only reveal hepatocellular damage without fibrosis. As the treatment with CCl_4 is continued, however, the animal gradually acquires a "tolerance" toward this poison, so that the adrenal cortex will respond less and less to each successive injection. Perhaps the final reason will be found in the damage which chronic poisoning with CCl_4 must cause in the adenohypophysis, so that the latter fails to maintain the adrenocortical response, or in the progressively decreasing liver function, which, because of nutritional or endocrine reasons, may inhibit the pituitary-adrenal "axis." The result, in any case, may be the removal of the factor which prevents the proliferation of the connective tissue elements and therefore the establishment of a fibrosis. The latter, in the early stages, is still "reversible," if an excess of adrenal steroids is supplied by treatment with cortisone. If, however, the development of the fibrous tissue is allowed to continue unhampered by an excess of endogenous or exogenous adrenal steroids, a stage will finally be reached when the "mature" fibrous tissue can no longer be affected. A hypothesis on these lines would account for the initial stage of hepatocellular damage without fibrosis, as well as for its gradual transition into the stage of reversible, and later irreversible, fibrosis without invoking an impairment of the regenerative capacity of the fibrotic liver, as is the case at present. The impaired regenerative capacity of the liver may well be, as has been suggested elsewhere,[33] the result and not the cause [2] of the fibrosis. In favor of this view would be the fact that liver cell proliferation can still be observed in the advanced stages of fibrosis (Fig. 7) and that the formation of fibrous tissue under the influence of CCl_4 is seen at least as early, if not earlier, in the livers of rapidly growing baby rats as in those of older animals, despite the presence of many mitotic figures.[34]

SUMMARY

The collagen content has been estimated in the liver of normal rats and of rats treated with carbon tetrachloride for periods of 14 and 31 weeks. The administration of cortisone to such animals led to a significant reduction in the amount of collagen present in the early stage of fibrosis but was without effect in the late stages. The histological findings were compared with the chemical determinations, and a better correlation has been found with the silver impregnation stain for reticulin than with Mallory's azocarmine stain. Cortisone significantly raised the fat content of the liver in the early but not in the late stage of fibrosis, when its administration was followed by a significant increase in the mortality of the animals. The part possibly played by the adrenal cortex in the development of experimental fibrosis of the liver has been discussed in the light of these findings.

Cortisone was supplied by the Medical Research Council. Cooperation in the preparation of this study was given by Dr. D. Darlington, who made the statistical analysis of the data; Dr. R. J. Boscott, who suggested the use of propanol for the determination of the dry weight of the liver samples and calcium pantothenate supplements; Miss R. H. Lawrence, who gave technical assistance; Mr. R. Varvarande, who prepared the reticulin stains, and Mr. W. J. Pardoe, who prepared the photomicrographs.

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STUDIES IN FIBROSIS OF THE LIVER INDUCED BY CARBON TETRACHLORIDE

III. Pantothenic Acid and Liver Fibrosis

K. ATERMAN, M.D., M.R.C.P., D.C.H.
BIRMINGHAM, ENGLAND

IT HAS repeatedly been shown that the hepatotoxic effects of acute and chronic poisoning with carbon tetrachloride (CCl_4) can be modified by various dietary components,[1] including members of the vitamin B complex, such as vitamin B_{12}.* Little work on these lines, however, appears to have been published on the possible effects of pantothenic acid, although several European workers have asserted that an excess of this vitamin can exert a favorable influence on the hepatic changes seen in certain experimental animals and in patients with liver disease. Jürgens and Pfaltz,[4] for instance, stated that in rats fed on a diet deficient in pantothenic acid fatty livers developed, which were restored to normal by supplying the missing vitamin. Jürgens [5] found that high doses of pantothenic acid antagonized in rats, rabbits, and guinea pigs the effects on the prothrombin time caused by bishydroxycoumarin (Dicoumarol), which in the doses given produced degenerative changes in the liver of the experimental animals. According to Glanzmann and Meier,[6] who cite Abelin [7] in support of their findings, pantothenic acid exerts a protective effect on the course of experimental thyrotoxicosis; the liver cells of animals given this vitamin appeared less atrophic and were less depleted of glycogen than were the liver cells of the thyrotoxic controls. Cavalcanti and Levis [8] similarly claim a reduction in the fat content of the liver of rats poisoned with phosphorus, if these animals were given pantothenic acid with or without inositol. Benda and Rissel [9] have shown that pantothenic acid considerably lessens the harmful effects of allyl formiate on the liver and suggested the use of this vitamin in the treatment of liver disease. Italian workers have, in fact, recently claimed an improvement in the liver function tests of their patients thus treated.† Interest in the effects of pantothenic acid was further stimulated by the studies of Ralli [10] on the relation of this vitamin to the adrenal cortex and the response of rats to "stress." Since, as has been suggested in the preceding paper, an altered function of the adrenal cortex may be one of the factors contributing to the development of the fibrosis of the liver, it was decided to see whether an excess of pantothenic acid could affect the poisoning with CCl_4.

METHODS

Ten female rats, purchased from the Agricultural Research Council (Compton Field Station) and ranging in weight from 93 to 139 gm., average 112 gm., were fed ad libitum the stock rat cake diet to which 0.1% of calcium pantothenate,‡ thoroughly mixed into the diet, had been

* References 2 and 3.

† References 10 and 11.

‡ The calcium pantothenate was supplied by Roche, Ltd.

added. They were given 29 subcutaneous injections of 0.1 to 0.12 ml. of CCl_4 over a period of 14 weeks, at the end of which time they were killed and the collagen content of the liver was determined. They were compared with a group of rats which were treated in an identical manner, except that the supplements of pantothenate were omitted. The results obtained in this group, as well as the details of the histological and chemical methods used, have already been described in the preceding paper.

RESULTS

The feeding of an excess of the pantothenate had little effect on the weight or on the appearance of the animals. Two rats died before the experiment was terminated, and in the livers of the two animals a definite fibrosis was present. In one of the remaining animals gross persistent ascites developed about two weeks before autopsy; free fluid was found in the peritoneal cavity in an additional rat at the end of the experiment. The liver in most animals was enlarged, appeared finely nodular with an irregular liver margin, and on cutting felt tough. Compared with the control

The Effect of Calcium Pantothenate on the Collagen Content of the Liver in Fibrosis Due to Treatment with Carbon Tetrachloride for Fourteen Weeks

Carbon Tetrachloride					Carbon Tetrachloride plus Pantothenate				
No. of Animal	Collagen, Mg. per Gm. Wet Liver	Total Collagen, Mg.	Mallory's Azocarmine Stain	Silver Stain	No. of Animal	Collagen, Mg. per Gm. Wet Liver	Total Collagen, Mg.	Mallory's Azocarmine Stain	Silver Stain
22	1.986	15.666	+	++	30	2.406	15.418	++	++
50	2.620	22.272	+	++	79	3.216	26.502	+++	+++
65	2.960	24.841	++	++	80	5.296	67.793	+++++	+++++
60	3.335	27.981	+++	+++	76	5.531	56.144	++++	++++
69	3.911	34.067	+++	+++	72	5.549	53.805	++++	++++
19	4.700	39.478	+++	+++	77	6.148	58.222	++++	++++
57	6.463	60.426	++++	++++	73	6.694	75.644	++++	++++
38	6.783	60.523	++++	+++	75	10.931	95.651	+++++	+++++
13	7.105	68.384	+++	++++		.....	.....		
56	9.944	93.974	++++	++++		.....	.....		
Mean	4.984 ± 0.795	45.661 ± 8.167			Mean	5.733 ± 0.90	56.149 ± 9.09		

group of animals without the pantothenate supplement, its average weight was slightly, but not significantly, higher (9.57 ± 0.70 gm. compared with 8.89 gm.); its fat content did not differ significantly from that of the normal control animals of the previously described experiment (4.99%).

On histological examination a definite fibrosis was found in most animals. Owing to the distribution of the fibrous tissue the lobular architecture was obliterated, and the hepatic parenchyma was divided into many nodules of varying diameter. By comparison with animals treated with CCl_4 only, the fibrosis in the groups also receiving pantothenic acid appeared more advanced, but the chemical estimation of the collagen content did not yield a significant difference (Table). This comparison illustrates the difficulty of assessing reliably differences in the severity of the fibrosis by histological means alone once the fibrosis has advanced beyond a certain stage. The experiment has since been repeated in another group of littermate rats belonging to an inbred strain used in this laboratory, in which the fibrosis produced was less marked. Littermate controls showed no difference in the histological appearance of the liver attributable to the feeding of supplements of pantothenic acid.

One animal (No. 30) must be singled out because of certain differences. It had been noticed that this rat, originally belonging to another group, grew more slowly

than the other animals, and it was therefore transferred after five weeks of treatment with CCl_4 alone to the group of animals receiving the supplements of pantothenic acid, as well. This, however, had no effect on the slow rate of growth, and so at the time of autopsy both the weight of the body (125 gm.) and the weight of the liver (6.18 gm.) were below the average weight for that group. Macroscopically the liver appeared smooth and could be cut with ease; the histological examination showed it to be the least-affected specimen of its group, an observation borne out by the chemical determination of the collagen content (Table).

COMMENT

It is generally accepted that an excess of pantothenic acid has no deleterious effects on the normal animal. Although the experiment described here was not prolonged into the advanced stage of liver fibrosis, it appears that an excess of this vitamin is also well tolerated by animals with a significant degree of liver damage; this recalls the observation by Gershberg, Rubin, and Ralli,[13] who gave doses of pantothenate far in excess of the daily requirements to patients with fibrosis of the liver.

The data presented here show that pantothenic acid does not materially affect the development of the fibrosis of the liver due to chronic poisoning with CCl_4. This is in agreement with the observation of Forbes, Leach, and Williams[14] that pantothenic acid has no protective effects against acute poisoning with CCl_4. It is apparent, therefore, that the "protective and anti-necrotic function" of pantothenic acid postulated by Banche and Daprà[11] does not apply to the hepatotoxic effects of CCl_4.

The milder fibrosis observed in the rat that failed to grow raises the question whether one of the factors determining an animal's susceptibility toward CCl_4 is its rate of growth, as has been suggested by Himsworth[15] for the experimental dietary injury to the liver. It has been stated that the rapidly growing liver cells of young rats are remarkably resistant toward CCl_4,[16] differing in that respect from the liver cells of puppies, but preliminary observations on very young rats treated with CCl_4 seem to suggest that fibrosis of the liver can be induced at least as rapidly, if not more so, in the fast-growing young rat as in the maturer animal.[17] The possibility can therefore not be excluded that the milder fibrosis observed in the animal described above was actually determined by its unusually slow rate of growth, which suggests a study of the effect of factors influencing growth on the course of the experimental liver fibrosis.

SUMMARY

Rats which were treated with repeated injections of carbon tetrachloride were fed an excess of calcium pantothenate throughout the period of CCl_4 administration. Neither the histological examination nor the determination of the collagen content of the liver showed a significant difference by comparison with animals given carbon tetrachloride only.

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HISTOCHEMICAL CHANGES IN IRRADIATED OVARIES

II. Carbohydrate and Lipid Localization

ALVAN G. FORAKER, M.D.
HOUSTON, TEXAS
SAM WESLEY DENHAM, M.D.
AND
M. HARLAN JOHNSTON, M.D.
JACKSONVILLE, FLA.

THE PRESENT communication records a continuation of histochemical studies in irradiated ovaries. A previous report was concerned with sites of dehydrogenase activity.[1] Areas of diminished dehydrogenase activity corresponded to areas of cell damage, as seen in conventional hematoxylin- and eosin-stained sections. In furtherance of this investigation, blocks of ovarian tissue from the same rabbits have been subjected to periodic acid and Sudan black B staining.

The purpose of this report is to record the changes induced in the ovary by irradiation as reflected by these histochemical techniques. In the present investigation, four weeks after administration of a tissue dose of 400 r to the left ovary, the irradiated ovaries of rabbits revealed diminution in the number of ova, change in appearance of the surviving ova, and lessening of response to hormonal stimulation. The atretic ova demonstrated a strong reaction to the periodic acid staining routine. There was little change in the staining reaction of the stromal cells.

MATERIALS AND METHODS

Twelve virgin female rabbits ranging from 6 to 8 weeks in age and from 1,180 to 3,140 gm. in weight were each given 400 r in air in a single dose to a 6.0 cm. external port over the left ovary. The factors of irradiation were constant potential 200 kv. at 18 ma., with a half-value layer of 1.2 mm. Cu. The distance was 50 cm., with a dosage rate of 50 r per minute. With these factors at a depth of 2.0 cm., the tissue dose to the ovary is approximately the same as the air dose.

Four weeks later six of the rabbits were given intravenous injections of urine from nonpregnant women, and six rabbits were given intravenous injections of urine from pregnant women according to a standard routine for the Friedman test, except that the second injection was given after a 24-hour interval.[2] Forty-eight hours after the first injection, the nonirradiated ovaries of all rabbits presented the classic appearance of either the negative (nonpregnant) or the positive (pregnant) Friedman test.[3] In contrast, the irradiated ovaries were smaller, paler, and firmer.

From the Departments of Pathology, Obstetrics and Gynecology, and Radiology, Emory University School of Medicine and Grady Memorial Hospital, Atlanta.

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Present addresses of the authors are The University of Texas, M. D. Anderson Hospital for Cancer Research (Dr. Foraker), St. Vincent's Hospital (Dr. Denham), Riverside Hospital (Dr. Johnston).

Blocks from each ovary, about 3 mm. in thickness, were fixed in neutralized formaldehyde solution (Formalin) and in Rossman's fluid (absolute alcohol saturated with picric acid, 90 cc., and formaldehyde solution, 10 cc.).[4] The Rossman fixed blocks were washed; dehydrated through alcohol, cedarwood oil, and xylene, and embedded in paraffin. Sections (5 μ) were stained with the periodic acid stain.[5] Control sections were saliva-digested to remove glycogen before stained.[6] Additional sections from these blocks were stained with hematoxylin and eosin for comparison.

Frozen sections (15 μ) from the formaldehyde solution-fixed tissues were stained with Baker's Sudan black B to demonstrate lipid and were counterstained with Bismarck brown R.[7] Additional frozen sections from these blocks were stained with hematoxylin and eosin.

RESULTS

In the right ovaries no effect of irradiation was seen. These ovaries from rabbits given injections with urine from nonpregnant women contained numerous ova in various stages of development and cystic follicles. The nonirradiated ovaries of rabbits given injections with urine from pregnant women also contained numerous ova in various stages of development, with corpora lutea and corpora hemorrhagica. In the irradiated ovaries comparatively few normal developing ova were found. Many of those that survived tended to have a pale shrunken appearance. Some retained a zona pellucida and follicle cells. In the irradiated ovaries from rabbits given injections with urine from nonpregnant women, little attempt at cystic follicle formation was found. In the ovaries from rabbits given injections with urine from pregnant women, an occasional abortive attempt at formation of a hemorrhagic cystic follicle was seen, but no florid corpora lutea or corpora hemorrhagica were demonstrated. The interstitial cells showed no change in cytologic pattern in the hematoxylin and eosin sections.

Comparison of test and saliva-digested control sections stained with periodic acid showed glycogen to be present only within developing ova and only in moderate amount. This was seen in the right ovaries from all rabbits (Fig. 1*B*). The primordial ova and other ovarian structures contained no demonstrable glycogen. In both test and control sections the zona pellucida showed intense dark red staining (Fig. 1*A* and *B*). The follicular liquid of the cystic follicles revealed a considerable, but somewhat less intense, red staining in both test and control sections. The basement membranes and reticular fibers were stained lightly pink. This staining was not affected by previous saliva digestion.

The irradiated ovaries of all rabbits had similar reactions to periodic acid staining (Fig. 2*A* and *B*). No glycogen was demonstrable in any portion of the ovaries. Ova which retained the usual appearance in hematoxylin and eosin sections also retained periodic acid-positive staining of their zona pellucida. Degenerating ova which had a granular appearance in hematoxylin and eosin sections had a strong red staining in the periodic acid sections unaffected by saliva digestion. This was the most striking feature of these sections. Ova which had progressed to hyalinization did not have this periodic acid staining reaction.

Sections of the nonirradiated ovaries subjected to Sudan black B had their most striking cytoplasmic lipid staining in the stromal cells. Cells of corpora lutea showed a less degree of staining, increasing in prominence as the corpora lutea approached maturity. Cells of atretic follicles and some follicle cells of primary ova had slight staining reaction. The zona pellucida reacted lightly to the lipid stain, and the developing ova had a fine black stippling (Fig. 3*A* and *B*).

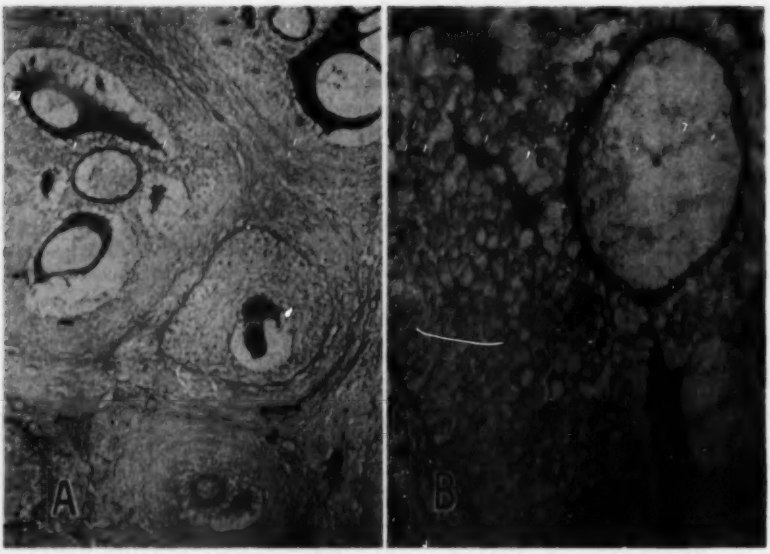

Fig. 1.—Right (nonirradiated) rabbit ovary from negative Friedman test. *A*, developing ova and cystic follicles. The zona pellucida is strongly stained, the follicular liquid less so. This reaction is not affected by previous saliva digestion; hence it is not due to glycogen. Periodic acid stain; × 75. *B*, detail of same section. Zona pellucida and follicular liquid stained as before. Very faint staining within ovum. This reaction is prevented by previous saliva digestion; hence glycogen was present within the ovum. Periodic acid stain; × 330.

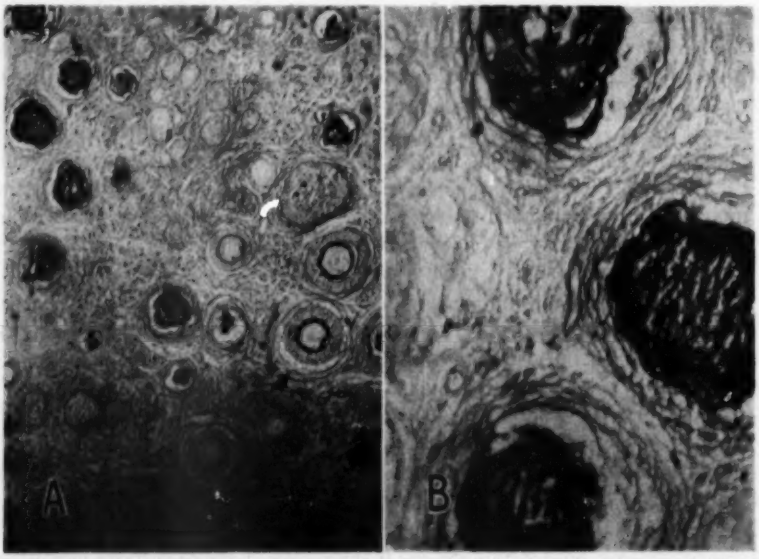

Fig. 2.—Left (irradiated) ovary from rabbit whose right (nonirradiated) ovary is illustrated in Figure 1. *A*, no developing cystic follicles are seen; hence ova are more numerous in section. Zona pellucida retains staining quality in ova, showing little irradiation effect. Atretic ova have strong staining reaction. Completely hyalinized ova show no reaction to periodic acid routine. Periodic acid stain; × 75. *B*, detail of same section showing several atretic ova. This reaction is not affected by previous saliva digestion; hence it is not due to glycogen. Periodic acid stain; × 330.

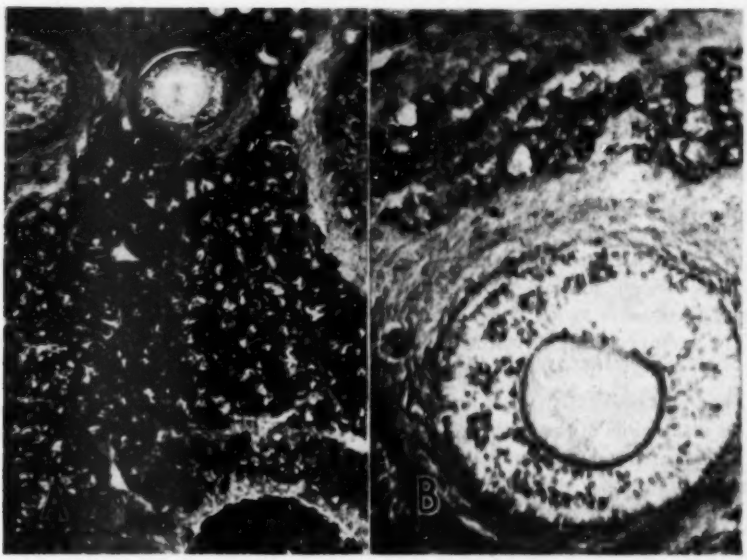

Fig. 3.—Right (nonirradiated) ovary from negative Friedman test. *A*, strong staining reaction of interstitial cells; lighter reaction of cells of developing ova and of atretic follicles. Sudan black B stain; × 75. *B*, detail of same section. The granulosa cells react very lightly to the lipid stain. The zona pellucida is moderately stained. Definite granules of sudanophilic material lie in the ovum. Sudan black B stain; × 165.

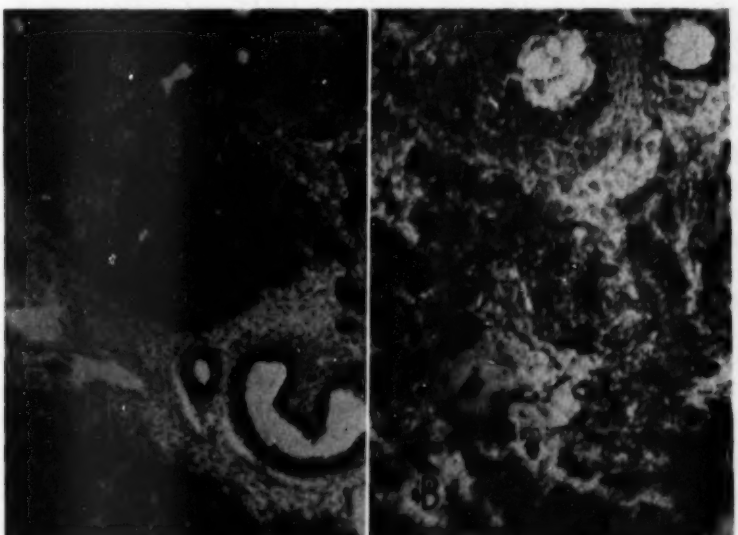

Fig. 4.—Left (irradiated) ovary of rabbit whose right (nonirradiated) ovary is illustrated in Figure 3. *A*, two-thirds of width of shrunken ovary, including hilum, is seen. Interstitial cells retain reactability with stain. Sudan black B stain; × 75. *B*, detail of same section. Ovum near top retains some stainable lipid and has lipid-containing follicle cells. Scarred refractile ovum near bottom has no viable follicle cells. Sudan black B stain; × 165.

The irradiated ovaries from all rabbits revealed little change in the intensity of cytoplasmic lipid staining of interstitial cells (Fig. 4*A* and *B*). Degenerating ova and other structures showed essentially no lipid staining. Ova with less morphologic evidence of damage retained faint reactability to Sudan black.

COMMENT

In several respects this study has reiterated the known phenomenon that the ova are more sensitive to radiation than are stromal cells.[6] Since the ova and adjacent follicle cells were so markedly damaged, it is not surprising that the ability of the ovaries to respond to hormonal stimulation was seriously compromised.

The staining reactions of the nonirradiated ovaries are consistent with published reports, especially those of Deane [4] and Harter.[5]

The histochemical changes in the irradiated ovaries merely reflect cellular damage, as discernible in the hematoxylin- and eosin-stained sections. No findings were ascribable as peculiar to radiation effect. The changes include disappearance of stainable lipid and glycogen in the ova. Those ova in the irradiated ovaries which retained the normal cytologic pattern also retained some reactability to Sudan black B. No glycogen could be seen in these ova, but the glycogen reaction of ova in nonirradiated ovaries was so weak that its absence does not justify special emphasis.

The most striking finding in the irradiated ovaries was the strongly positive periodic acid staining reaction of atretic ova. This is probably also a function of general deterioration, rather than a specific radiation change. Harter [9] described glycoproteins reacting similarly in atretic ova which resisted various types of enzyme digestion. The periodic acid-leucofuchsin method, as employed in this study, will visualize all polysaccharides and protein- or lipid-bound reactive carbohydrate (i. e., glycogen, glycoprotein, and glycolipid).[9] We can only say on the basis of saliva digestion that the material in the atretic ova in this study was not glycogen. Harter [9] employed a battery of extractives, enzymes, and other reagents in labelling a similar substance in atretic ova as glycoprotein.

Retention of lipid-staining properties of the interstitial cells is consistent with the absence of change in their cytologic pattern. It correlates with the retention of dehydrogenase activity of these cells described previously.[1]

Histochemical demonstration of sites of dehydrogenase activity, glycogen and other periodic acid-positive substances, and lipid, as seen in these studies, has merely elaborated findings discernible in conventional hematoxylin and eosin sections. With the radiation dosage and time interval employed, there is no evidence that these histochemical techniques have revealed different or more widespread patterns of radiation damage than those previously described.[8]

SUMMARY

The left ovaries of 12 rabbits were subjected to 200 kvp irradiation for a single dose of 400 r. Four weeks later six of the rabbits were used in negative and six in positive Friedman tests. Tissues from all ovaries were stained with the periodic acid stain to demonstrate glycogen and other periodic acid-positive substances and with Sudan black B to demonstrate lipid. The results showed little evidence of irradiation damage of the stromal cells either in pattern or in lipid content; obliteration of many of the ova in irradiated ovaries, those remaining being largely

atretic with no stainable lipid and with a considerable deposition of a periodic acid-positive substance, not glycogen; little evidence of ability of the irradiated ovaries to respond to the hormonal stimulation of the Friedman test.

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EFFECT OF INORGANIC PHOSPHATE ON DEVELOPMENT OF HEMOGLOBINURIC NEPHROSIS IN RABBITS

JOSEPH J. LALICH, M.D.
MADISON, WIS.

LUCKE [1] and Foy and co-workers [2] have evaluated most of the known theories that have been proposed to explain the pathogenesis of lower nephron nephrosis, crush injury, transfusion anurias, or hemoglobinuric nephrosis. They have clearly delineated both the clinical and the pathological aspects of the disease. Their discussions of pathogenesis, as well as those of others,* have raised questions as to the relative importance of the different variables. The role of aciduria in the development of hemoglobinuric nephrosis remains a highly controversial question.†

Experimental evidence has shown that related factors may be of more significance in the production of hemoglobinuric nephrosis than the hemoglobinemia per se.‡ The known associated factors which predispose to hemoglobinuric nephrosis when combined with injections of oxyhemoglobin are antecedent tubular injury,§ starvation and water restriction,‖ injection of histamine [11] or arsine,[12] and injection of methemoglobin into animals with aciduria.¶ Many of the experimental studies have been criticized because the artificial experimental conditions were so dissimilar to the disease as it is encountered in man.[15] To overcome such objections it is necessary that, in addition to the production of the anatomical lesions and altered renal physiology, physiological and chemical alterations more closely simulating those encountered in man be produced. It seemed advisable to study the influence of phosphate excretion on the production of hemoglobinuric nephrosis for several reasons. Increased excretion of inorganic phosphate may occur during acidosis and starvation,[16] conditions frequently associated with the development of this syndrome. Phosphoric acid, along with other substances which are released from injured muscles, has been implicated as a factor in the production of nephrosis.[17] Lastly, it has been possible to produce nephrosis by feeding massive doses of inorganic phosphate to rats.[18]

From the Department of Pathology, University of Wisconsin Medical School.

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*References 3 and 4.

†References 2, 5, 6, 7, and 8.

‡References 7, 8, 9, 10, and 11.

§References 8, 9, and 10.

‖References 10 and 11.

¶References 13 and 14.

METHOD

New Zealand white rabbits of both sexes weighing from 2.1 to 3.9 kg. were used. The oxyhemoglobin solutions and the oxidizing salts were prepared as in a previous study.[14] Three experiments, A, B, and C, were performed to show the effect of phosphate alone and in combination with oxyhemoglobin or methemoglobin.

In experiment A the phosphate concentration in the plasma and urine was determined in five pellet-fed# rabbits which were subjected to three days without water and five days of starvation. In a repeat study five weeks later on the same animals, withholding of food and water was combined with an intraperitoneal injection of 1% buffered sodium phosphate equal to 0.25 gm. per kilogram of body weight on the second and third days of starvation. The observations made in this experiment suggested the concentrations at which one might produce a phosphate nephrosis. One of three diets was fed to 22 animals in Experiments B and C for at least two weeks prior to the injection of oxyhemoglobin or methemoglobin. Some were fed rabbit pellets, and a few received ground oats with 1% NaCl; whereas most were given 1% sodium phosphate mixture in oats. The ratios of mono- and disodium phosphate mixtures varied from 1:1 to 3.5:1 parts, respectively, to attain the desired urine pH.

In experiment B, 11 rabbits which were given oxyhemoglobin were fed three diets.

In experiment C, 11 rabbits were fed only oats and 1% sodium phosphate mixture prior to the injection of methemoglobin. The methemoglobin was produced in vivo by combining an intravenous injection of oxyhemoglobin with an intraperitoneal administration of buffered 1% sodium nitrate or 1% potassium ferricyanide. All of the rabbits given intravenous oxyhemoglobin received 1 gm. per kilogram of body weight in three doses in six to eight hours. Following each of the above procedures, the plasma and urine phosphate concentrations were determined.[19] The phosphate in the plasma and urine is expressed in milligrams of phosphorus (P). To ascertain whether renal injury was induced, nonprotein nitrogen concentrations were determined either on blood obtained at autopsy or drawn from the heart five days after injection. The nonprotein nitrogen values were determined with a micro-Kjeldahl method.[20] The autopsies were performed from the first to the eighth day. The tissues were examined, fixed in 10% formalin, embedded in paraffin, and sectioned. The pigment-cast counts shown in the Tables represent the total number observed in 10 low-power fields.

RESULTS

Experiment A.—Data pertaining to inorganic phosphate excretion during starvation and the withholding of water alone and in combination with an intraperitoneal injection of buffered sodium phosphate are shown in Table 1.

Normal values of phosphorus in plasma in these animals varied from 4.1 to 6.0 mg. per 100 cc. Animals fed rabbit pellets seldom excrete more than 15 mg. of phosphorus each day (Table 2). Three days without water and five days of starvation raised the plasma phosphorus levels slightly in two of five animals. The inorganic phosphate excretion in the urine, however, is markedly increased. During starvation, as one would anticipate, all the animals developed aciduria. When the experiment was repeated and the withholding of food and water was combined with an intraperitoneal injection of buffered sodium phosphate, the alterations in phosphate metabolism were more marked. The plasma phosphorus levels were slightly higher than previously on the fifth day of starvation in two of five rabbits. Under these circumstances three rabbits excreted more than 200 mg. of phosphorus in one day. The urine pH was not as acid, due to the buffering effect of the disodium salt administered intraperitoneally. Unlike the observations following starvation and water deprivation alone, two of five rabbits now had slight increases in plasma nonprotein nitrogen. At autopsy all kidneys were paler than usual. Microscopic

#Master Mix Rabbit Pellets (McMillen Feed Mills, Division of Central Soya Company, Inc., Fort Wayne, Ind.).

examination revealed many eosin casts, focal lymphocytic infiltration, or vacuolar degeneration of epithelial cells in the proximal convoluted tubules. Renal dysfunction, as manifested by an elevated plasma nonprotein nitrogen or gross alterations of the kidneys, can be induced by starvation and intraperitoneal injection of buffered sodium phosphate. An explanation for the lack of correlation between increased plasma phosphate concentrations and the excretion of phosphate in the urine is not evident from this study.

Experiment B.—Results observed in 11 rabbits following injection of oxyhemoglobin are shown in Table 2.

Three animals were fed rabbit pellets, and two were given oats with 1% NaCl; whereas six received a mixture of 1% sodium phosphate salts in ground oats. Plasma phosphate was slightly increased in two of six rabbits which received

TABLE 1.—*Studies Following Starvation Alone and Starvation Combined with Intraperitoneal Injection of Sodium Phosphate* *

Rabbit No.	Sodium Phosphate Injected Gm./Kg.	Plasma P, 5th Day, Mg./100 Cc.	Plasma Nonprotein Nitrogen, 5th Day, Mg./100 Cc.	P Excreted† and Urine Volume, Mg. P/Ml. Urine	Lowest Recorded Urine pH	Autopsy After P Injection, Days	Combined Kidney Wt., Gm.	Kidney
1	0	4.7	38	110/135	5.3	..	....	.....
2	0	6.3	35	90/119	5.5	..	....	.....
3	0	5.9	35	213/336	5.6	..	....	.....
4	0	7.1	31	64/164	6.0	..	....	.....
5	0	7.6	37	98/96	5.0	..	....	.....
1	0.5	4.9	26	235/128	5.2	4	15.5	Eosin casts, lymphocytes
2	0.5	9.5	94	69/150	6.3	4	19.0	Eosin casts, lymphocytes
3	0.5	5.0	28	211/102	7.3	4	14.1	Eosin casts, infarct
4	0.5	4.4	26	335/210	6.3	4	14.3	Eosin casts
5	0.5	10.3	70	234/198	7.0	4	14.7	Lymphocytes, eosin casts

* Rabbits were fed rabbit pellets prior to starvation. Food was withheld for five days, water for 3 days. The buffered (pH 7.0) 1% sodium phosphate was injected intraperitoneally on the second and third days. Urine was collected on the third, fourth, and fifth days.

† Values in Table represent the highest P excretion observed during 24 hours.

phosphate in the diet. The urine pH, depending on the diet and the salt mixture, fluctuated over a wide range from 5.3 to 8.0. The inorganic phosphate excretion was negligible in rabbits fed pellets or oats and NaCl. Animals which received mixtures of mono- and disodium phosphate in the diet excreted much more phosphorus in the urine. Following injection of oxyhemoglobin, none of these rabbits developed significant increases of plasma nonprotein nitrogen or retained many pigment casts. Two rabbits (Nos. 8 and 10) given an intraperitoneal injection of buffered sodium phosphate (0.25 gm. per kilogram of body weight), along with intravenous oxyhemoglobin, died soon after the first injection. Only one of eight rabbits had an appreciable increase in kidney weight; however, very few pigment casts were found in the kidneys. We wish to point out that in phosphate-fed rabbits no more than 75 pigment casts were seen in 10 low-power fields, in contrast to the greater numbers observed in the next group of animals fed a similar diet but receiving methemoglobin instead of oxyhemoglobin.

Experiment C.—The combined effect of in-vivo-produced methemoglobin and feeding a 1% mixture of sodium phosphate salts in oats on the development of hemoglobinuric nephrosis in 11 rabbits is shown in Table 3.

Only slight increases in plasma phosphorus were encountered in this group. The urine pH fluctuated widely, depending upon the concentration of the disodium phosphate which was included in the 1% phosphate salt mixture. The concentrations of oxidizing salt shown in Table 3 represent the total which was administered in three divided doses. The larger rabbits at the time of oxyhemoglobin injection were given greater concentrations of oxidizing salts. The urine phosphate levels during the time when the methemoglobin was excreted were high. In contrast to the urine excretion in the previous two groups of animals, anuria was encountered for the first time in 3 of 11 rabbits. The plasma nonprotein nitrogen on the fifth day was 70 mg. per 100 cc. or more in 3 of 11 rabbits. Two animals died, one following the first injection of sodium nitrite and oxyhemoglobin and the other on the third day. In the first rabbit there was a marked decrease in systolic pressure and a profound cyanosis prior to death. Whether death was induced by peripheral vasodilatation

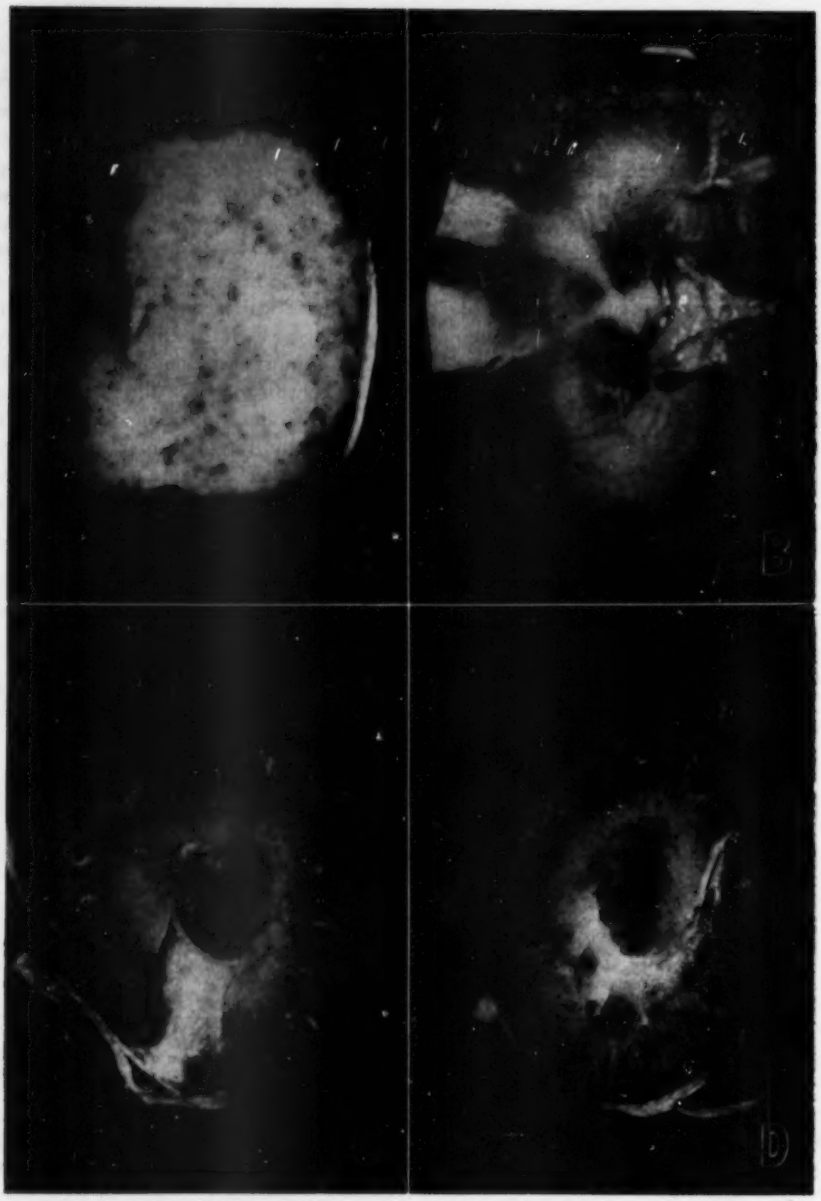
TABLE 2.—*Relationship Between Urine Phosphate Excretion and Intravenous Injection of Oxyhemoglobin*

Rabbit No.	Diet	Plasma P Before Injection, Mg./100 Cc.	Urine pH Before Injection	P Excreted 1st Day, Mg. P/Ml. Urine	Plasma Nonprotein Nitrogen, 5th Day, Mg./100 Cc.	Autopsy, Days	Combined Kidney Wt., Gm.	Total Pigment Casts*
6	Pellets	...	8.0	5/172	52	8	12.7	2
7	Pellets	...	8.0	4/162	34	8	14.2	0
8	Pellets	5.2	...	.....	31	1†	16.2	0
9	Oats, 1% NaCl	4.6	8.0	12/118	32	8	14.8	0
10	Oats, 1% NaCl	4.1	6.1	.....	30	1†	13.7	5
11	Oats, 1% sodium phosphate	Did not eat, 8.3	6.4	15/166	34	8	23.1	29
12	Oats, 1% sodium phosphate	6.4	5.3	83/110	31	8	12.2	11
13	Oats, 1% sodium phosphate	5.4	5.8	147/122	22	8	10.2	2
14	Oats, 1% sodium phosphate	6.0	8.0	136/147	32	8	12.6	28
15	Oats, 1% sodium phosphate	6.6	Acid	.....	50	8	12.3	74
16	Oats, 1% sodium phosphate	7.4	6.3	35/21	29	8	16.6	10

* Pigment casts counted in 10 random low-power fields of the renal cortex.

† Animals which were also given buffered sodium phosphate (0.25 gm. per kilogram of body weight) intraperitoneally died shortly after injection. Each rabbit received a total of 1 gm. per kilogram of body weight of oxyhemoglobin intravenously, except the two animals that died.

or the methemoglobinemia is not evident. Four rabbits had kidneys weighing more than 20 gm. In contrast to the six phosphate-fed animals which received oxyhemoglobin, many more pigment casts were observed following injection of oxyhemoglobin and oxidizing salt. Another significant observation is that Rabbits 24 and 27 retained many pigment casts, despite the fact that the urine was alkaline during the period of methemoglobin excretion. This is in contrast to a previous observation in pellet-fed rabbits which received intravenous methemoglobin.[14] The retention of pigment casts following injection of methemoglobin in one group of animals with alkalinuria and not in another is directly related, we believe, to the increased urinary excretion of inorganic phosphate in the present group of rabbits.



A and *B*, longitudinal sections of kidneys of Rabbit 25 after fixation in 10% formalin containing 1% HCl for 24 hours. This animal received intraperitoneal potassium ferricyanide, along with intravenous oxyhemoglobin. At autopsy eight days later there was a segmental accumulation of brown pigment, mostly in the renal cortex, which only developed a suggestion of Prussian blue color after acid formalin fixation. *C* and *D*, cross sections of kidneys from Rabbit 24. This rabbit had a marked elevation of plasma nonprotein nitrogen and died three days after the intraperitoneal administration of potassium ferricyanide and intravenous oxyhemoglobin. At autopsy there was a diffuse brown pigmentation of the cortex. Following fixation in acid formalin the renal cortex assumed an intense Prussian blue color, which is not evident in this photograph. The dark foci evident in the cortex are collections of brown pigment casts which did not change color. The interesting difference between these two rabbits is that it was possible to demonstrate more ferric iron and brown pigment in the cortex of the rabbit which died in uremia.

COMMENT

The methemoglobin employed by Bing [13] and ourselves in a former study [14] was formed in vitro prior to injection. Such studies raise, therefore, the question of whether equivalent renal injury would develop when the methemoglobin is formed in vivo. The efficacy of in-vivo-formed methemoglobin in this study suggests that the mechanism which inhibits the oxidation of oxyhemoglobin can be overcome. In addition to forming methemoglobin, sodium nitrite produces an immediate vasodilatory effect: whereas the ferricyanide, following its reduction in the body, is capable of forming Prussian blue during fixation in acid formalin. In view of the marked effect which methemoglobin exerts in the development of experimental hemoglobinuric nephrosis, the role of this pigment in the development of a similar syndrome in man should be evaluated. Several facts justify such an opinion. Methemoglobinemia does occur in man *; furthermore, amino acid metabolites,[23]

TABLE 3.—*Effect of Urine Phosphate and pH on Retention of Pigment Casts in Rabbits Excreting Methemoglobin* *

Rabbit No.	Plasma P Before Injection, Mg./100 Cc.	Urine pH Before Injection	Oxidizing Salt Injected, Mg./Kg.†	P Excreted 1st Day, Mg. P/Ml. Urine	Plasma Nonprotein Nitrogen, 5th Day, Mg./100 Cc.	Autopsy, Days	Combined Kidney Wt., Gm.	Total Pigment Casts
17	8.1	6.9	45 A	118/184	24	8	20.2	86
18	5.8	Acid	75 A	130/113	36	8	11.0	130
19	5.8	Acid	75 A	194/89	72	8	12.6	195
20	6.3	5.5	75 A	Anuria 3 days	94	8	24.3	589
21	7.2	6.9	25 A	.....	..	1‡	12.6	3
22	7.2	6.7	37 A	165/64	36	8	18.0	74
23	6.2	5.8	75 B	28/42	52	8	11.2	350
24	5.1	7.7	75 B	Anuria 3 days	240	8‡	32.8	526
25	5.4	6.5	75 B	Anuria 2 days	50	8	17.1	714
26	6.8	7.5	47 B	128/108	29	8	17.4	15
27	7.5	8.0	45 B	26/82	38	8	22.7	280

* All rabbits were fed ground oats plus 1% mixed sodium phosphate salts. Oxyhemoglobin and oxidizing salts were administered in three injections in six to eight hours.
† A, 1% sodium nitrite, pH 7.0; B, 1% potassium ferricyanide, pH 7.0.
‡ Animal died.

derivatives of sulfonamides,[24] and urochrome [25] are all capable of oxidizing oxyhemoglobin.

Stoner and Green have advanced an interesting hypothesis stating that normally occurring innocuous intracellular constituents may become toxic to the organism merely by virtue of becoming extracellular.[26] Both oxyhemoglobin and inorganic phosphate exist in sufficient concentration to exert a deleterious effect, providing they are released from the cell. Bywaters has shown that 75% of the inorganic phosphate is released from the injured muscles of patients with crush injury.[27] It appears doubtful that inorganic phosphate which is released from 5 to 10 kg. of injured muscle in man is capable of producing tubular injury alone. MacKay and Oliver had to feed massive doses of inorganic phosphate before producing nephrosis in rats.[18] The observations made in rabbits fed phosphates in oats (Experiment B) suggest that nephrotoxic phosphate concentrations were not employed. In keeping with Dunn's suggestion the increased urine phosphates exert some effect [17] and probably predispose indirectly to the renal injury. Schiess and co-

*References 21 and 22.

workers [28] and McCrory and co-workers [29] have shown that under increased loads of phosphate filtration the renal tubular mechanisms become saturated so that the excess phosphate is excreted in the urine. In agreement with these observations it was possible to demonstrate that animals fed phosphate salts excreted from 5 to 10 times more phosphorus in the urine than rabbits fed pellets or oats and sodium chloride. By feeding a 1% alkaline sodium phosphate mixture in ground oats and injecting oxidizing salts with oxyhemoglobin, it is possible to develop hemoglobinuric nephrosis in rabbits with alkalinuria. To my knowledge this is the first time hemoglobinuric nephrosis has been produced in animals with alkalinuria. It is noteworthy to add that, when increased urinary excretion of inorganic phosphorus is combined with injection of oxyhemoglobin, only insignificant numbers of pigment casts are encountered. That the simultaneous excretion of inorganic phosphate and methemoglobin in urine is capable of promoting pigment cast formation and affecting renal function is quite unusual, especially since it was neither necessary to starve the animals nor to deprive them of water prior to the injection of methemoglobin. Despite the fact that it has been possible to develop hemoglobinuric nephrosis in animals with alkalinuria, aciduria with its physiologic and biochemical changes is, in my opinion, probably more frequently encountered during the development of this syndrome in man.

SUMMARY

The influence of withholding food and water alone and in combination with an intraperitoneal administration of buffered sodium phosphate on the excretion of phosphorus in urine was established in five rabbits. The excretion of inorganic phosphate was greater when starvation and withholding of water were combined with an intraperitoneal injection of sodium phosphate. Eleven rabbits which were fed three different diets received intravenous oxyhemoglobin. Relatively few pigment casts were observed in the tubules, even though six of these animals were fed a 1% sodium phosphate mixture. When phosphate feeding was combined with an injection of methemoglobin in 11 other rabbits, many pigment casts were observed, irrespective of the urine pH. These findings suggest that increased excretion of inorganic phosphate in the urine may be an important related factor in the production of hemoglobinuric nephrosis in the presence of a methemoglobinemia.

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DYNAMICS OF INFLAMMATION AND OF REPAIR

IV. Chemotactic Substances in Normal Tissues

VIRGIL H. MOON, M.D.
C. G. GRAND
AND
GEORGE A. TERSHAKOVEC, M.D.
CORAL GABLES, FLA.

THIS subject was dealt with in a previous report by two of us.[1] A new capillary tube technique for observing chemotaxis was described, and the results of several hundred tests were given. The conclusion was drawn that normal animal tissues contain substances which attract neutrophiles in vitro. Subsequent evidence and attempts to reproduce the previous results convinced us that the technique then employed [1] is not reliable, and we do not recommend its use. Further investigation of the problem led to the experiments to be described.

The chemotactic effects of various micro-organisms, also many organic and inorganic substances, had been tested (Wells [2] and McCutcheon,[3] reviews), but a few reports had appeared on substances derived from normal or injured tissue cells. Yet leucocytic migration into the injured tissues is prompt and speedy. Moon [4] had noted the rapid migration of leucocytes into blister fluid after burns under aseptic conditions in man. Grand and Chambers [5] had reported that muscle fibers in tissue culture attracted leucocytes if mildly injured mechanically. Silverman [6] had shown by McCutcheon's [7] coverslip technique that the cells of human skin contain a water-soluble substance which strongly attracts leucocytes. The desirability of exploring the chemotactic properties of products derived from normal tissues is evident. Accordingly, we have made further investigations by techniques used previously by others.

METHODS

Data were collected by three different methods, the first two of which are modified tissue culture techniques. Each method is described in detail, after which the results are recorded.

Method 1 (Microcapillary Tube Method).—This technique was described previously.* A clean glass tube was drawn out in a gas flame to a capillary tube about 0.5 mm. in diameter. This was again drawn in a microflame to a diameter of about 0.1 mm. and several centimeters long. An extract of tissue to be tested for chemotactic effects was introduced by dipping the tip of the microcapillary tube into the solution, which entered by capillarity. The exposed tip was sealed by dipping into beeswax. A suitable length, about 3 mm., of the microcapillary tube,

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* References 5 and 8.

was cut off with a flat-faced forceps. The cut microcapillary tubes were placed on a moist filter paper in a Petri dish and used immediately after their preparation. The entire procedure was carried out under sterile conditions.

Tissue Extracts. A weighed quantity of each tissue was suspended finely in four volumes of 0.9% NaCl solution by means of a Waring Blendor. After autolysis at room temperature for two to three hours, the suspension was centrifuged; the supernatant fluid was adjusted to pH 7, clarified by centrifugation at high speed, and sterilized by filtration (Seitz). The extracts were then dried from a frozen state in vacuo and were sealed in glass ampoules until used. Regularly the extract from 1 gm. of tissue was dissolved in 0.5 ml. of Tyrode's solution. Extracts of rabbit lung, spleen, kidney, liver, brain, and muscles were made and tested.

Two sources for leucocytes were used: (1) The leucocytes of blood were obtained from the gray layer (buffy coat) which collects in centrifuged blood between the supernatant plasma and the underlying red blood corpuscles. This was secured by removing the plasma and dropping

Fig. 1.—The capillary tube (*center*) contains extract of rabbit liver. The explant (*left*) is from the buffy coat of rabbit blood. Note the swarming of leucocytes about the capillary tip. Culture 24 hours; × 25.

a small amount of embryonal juice on the gray layer. The resulting clot was lifted off, rinsed free of red blood cells, and cut into fragments for implantation in the culture. (2) Leucocytes from splenic tissues were obtained from chick embryos of various ages.

The Maximow double coverslip tissue culture method was used. The medium consisted of the following mixtures: for fowl leucocytes, equal parts of 25% chick embryo extract and of fowl plasma; for rat or rabbit leucocytes, equal parts of rat or rabbit serum and of the embryonal extract. An explant of clot or of splenic tissue containing leucocytes was made into this medium. The capillary tube was then placed tangential to the explant, with the open end 0.5 mm. distant from it. The preparation was then incubated at 37 C. Leucocytes migrated to the open end of the capillary tube, clustered about it, or entered the lumen, if the tissue extract had chemotactic properties (Fig. 1). A total of 87 tests was made by this method.

Method 2 (Method for the Study of Tissue Substance).—Tissue fragments measuring from 1.5 to 2.0 mm. were planted in tissue culture medium 0.5 mm. distant from the explant containing the leucocytes. All the preparations were incubated at 37 C.

During incubation the leucocytes migrated out of the explant in all directions and moved toward the test tissues in large numbers, if the latter contained attractive substances. Positive results usually were evident within 6 to 12 hours; observations were recorded at 12 and 24 hours. Many of the cultures were fixed and stained for permanent records. Often, bits of different tissues were planted in the same culture; this permitted observation under identical conditions for purposes of comparison (Fig. 2). A total of 354 tests were made by this technique.

Method 3 (Slide and Coverslip Method).—This is the technique developed by McCutcheon and his associates. It consists in placing upon a slide a minute target of the substance to be tested. A drop of fresh leucocytic suspension in plasma is applied; then a coverslip placed on the preparation is sealed on with white petroleum jelly (Vaseline) to prevent evaporation. The originators of this method made observations through a microscope having a warm stage and camera lucida attachment for tracing the movements of individual leucocytes. A variation of this method consisted in making a number of such preparations, incubating at 38 C., and noting

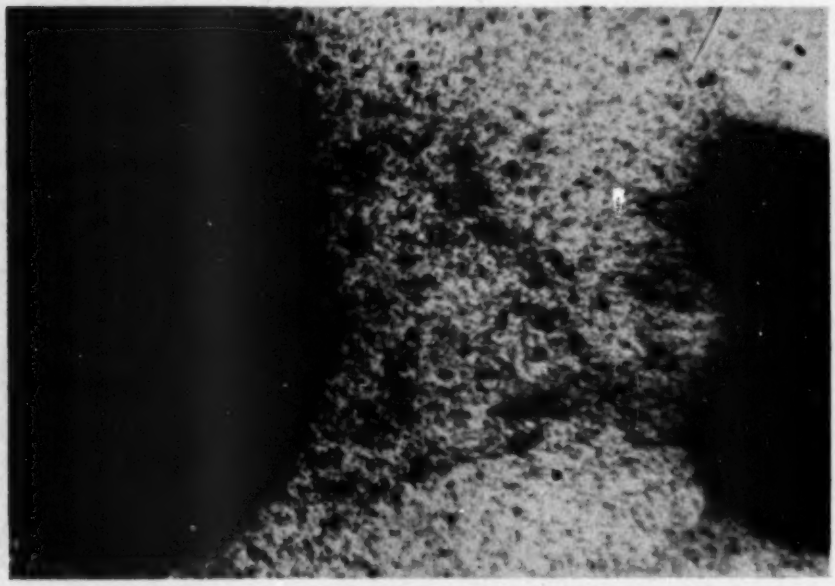

Fig. 2.—The explant (*left*) is from chick embryo spleen; the explant (*right*) is from bruised rat muscle. Note the migration of leucocytes toward the muscle. Culture 24 hours; × 50.

the clustering of leucocytes about the targets after varying periods of time, usually 3 and 18 hours. In order to test the response of the leucocytes to attraction, a target of killed and washed staphylococci was placed on the slide at a distance from the tissue target.

Scrupulous cleanliness of all glassware, including slides, coverslips, syringes, tubes, pipettes, etc., is essential. Glassware is taken from cleaning fluids, washed in hot tap water, then washed in distilled and in doubly distilled water, then washed in alcohol-ether, then dried. New slides and coverslips were used regularly; after cleaning, these were polished with soft cloth, then flamed, and stored in a covered jar. Cleanliness cannot be overemphasized; infinitesimal traces of chemicals will affect the motility of leucocytes.

Normal tissues were taken from a freshly killed rabbit and placed immediately in deep freeze until used. Leucocytes were obtained by injecting 100 ml. of 0.9% NaCl into the peritoneal cavity of a rabbit and withdrawing it several hours later. This suspension was then centrifuged at moderate speed; the supernatant fluid was drawn off, and the leucocytes were suspended in fresh plasma. A drop of this suspension was placed upon targets prepared as described, then

covered, sealed, and incubated at 38 C. Plasma was obtained by drawing rabbit blood from the heart into a dry cold syringe, transferring it immediately into paraffin-coated chilled tubes, then centrifuging at high speed in a low-temperature centrifuge at 0 to 1 C. These precautions usually prevent clotting of the plasma.

The results were observed microscopically after 3 and 18 hours' incubation. A definite accumulation of leucocytes at the margins of the target was recorded as positive (1+); very numerous leucocytes about the target were marked 2+. Dense accumulations and penetration of the target by leucocytes were recorded as 3+ (Figs. 3 and 4). This technique was used in 324 tests.

RESULTS

The results from tests for chemotaxis by each of the three methods are presented in tabular form.

TABLE 1.—*Extracts of Tissues Tested for Chemotaxis in Capillary Tubes*

Tissue	Tests	Negative	+	++	+++
Liver	19	6	0	6	13
Lung	20	8	0	1	11
Spleen	12	0	0	6	6
Brain	12	12	0	0	0
Kidney	12	12	0	0	0
Muscle	12	12	0	0	0

TABLE 2.—*Tissue Substance Tested in Culture Medium*

Tissue from Rat	Tests	Negative	+	++	+++
N muscle *	54	54	0	0	0
B muscle *	54	0	0	0	54
Lung *	18	0	0	18	0
Liver *	18	0	18	0	0
Kidney *	18	18	0	0	0
Tissue from 14-day chick embryo					
Muscle *	24	0	0	0	24
Lung *	24	0	0	24	0
Liver *	24	0	23	1	0
Kidney *	24	24	0	0	0
Muscle †	24	24	0	0	0
Lung †	24	0	0	24	0
Liver †	24	0	24	0	0
Kidney †	24	24	0	0	0

* Leucocytes from chick embryo spleen.
† These chick embryo tissues were tested with leucocytes from the buffy coat of chicken blood.

With leucocytes from chick embryo spleen, six tests on liver and eight on lung tissues were negative, but these extracts were strongly positive when tested with leucocytes from the buffy coat of fowl and of rabbit blood.

Extracts of brain, kidney, and muscle were uniformly negative, regardless of the source of the leucocytes.

In testing tissue substance for chemotaxis, an interesting difference was found between normal and injured muscle substance. A muscle in the leg of a rat was bruised; then 15 minutes later explants were made of both bruised and normal muscle into a tissue culture medium. These were tested as described under Method 2. The normal and bruised tissues are designed as *N* muscle and *B* muscle, respectively, in Table 2.

In these tests it is notable that bruised rat muscle was strongly chemotactic for chick embryo leucocytes (Fig. 2); whereas normal rat muscle was not. Contrasted with this, embryo chick muscle was strongly chemotactic to chick embryo leucocytes but not to those from the buffy coat of chicken blood. Kidney tissue from either the rat or the chick embryo did not attract leucocytes from either source.

A comparison of results shown in Tables 1 and 2 with those in Table 3 shows a few differences which require comment. The tests on kidney substance and on extract of kidney by Methods 1 and 2 were uniformly negative; whereas kidney substance showed definite chemotactic effects in tests by Method 3 (Fig. 4). In the former the leucocytes and tissues were heterologous (they were from different species); whereas in the latter both leucocytes and tissues were from rabbits.

A variation occurred also in muscle tissue as tested by Methods 2 and 3. Bruised rat muscle strongly attracted leucocytes from chick embryo spleen, as did also chick embryo muscle. Normal rat muscle did not attract these leucocytes, and chick embryo muscle failed to attract leucocytes from the buffy coat of chicken blood. On the other hand, rabbit leucocytes were consistently and strongly attracted to rabbit muscle in tests by Method 3 (Fig. 3).

TABLE 3.—*Tests for Chemotaxis by the Coverslip Technique*

Tissue	Tests	Negative	Positive	+	++	+++
Lung	54	2	52	22	22	8
Liver	48	7	41	18	16	7
Spleen	34	5	29	18	9	2
Skin	44	6	38	26	4	8
Testes	38	5	33	6	12	15
Kidney	42	3	39	12	10	17
Muscle	34	2	32	8	22	2
Brain	30	10	20	11	6	3

There was a similar lack of agreement in the tests on brain. Extracts of brain substance gave negative results uniformly (Table 1), but brain substance showed many strongly positive chemotactic reactions (Table 3). The agents used in the former were from different species of animals; in the latter they were homologous. We have no other plausible suggestions as to these variations and must be content to record the results as obtained by each technique.

Variations in the activity of the leucocytes were evident throughout the series made by Method 3. On one day the leucocytes migrated rapidly, giving a strongly positive reaction about the targets both of tissue and of staphylococci. On another day there were sluggish motility and negative or slightly positive responses to the targets, although technical details were identical on the two days.

Recently Ketchel and Favour [9] demonstrated that some factor residing in the plasma of different individuals caused marked variations in the migration of leucocytes in vitro. Leucocytes from the same source, when placed in one plasma, migrated several hundred per cent more than in another plasma similarly prepared. This finding supplies a plausible explanation for many of the variations in the results shown in Table 3.

For example, on one day, 10 tests on brain substance gave the following results: none negative, two positive, five strongly positive (2+), and three very strong (3+). On this day the staphylococci targets gave strong (2+) or very strong (3+) results. On two other days, 20 tests gave the following results: 10 negative, 9 positive (1+), and one strongly positive (2+). The Staphylococcus controls, on

Fig. 3.—Chemotaxis (3+) of rabbit muscle (*right*) for rabbit leucocytes. Incubation 18 hours; × 115.

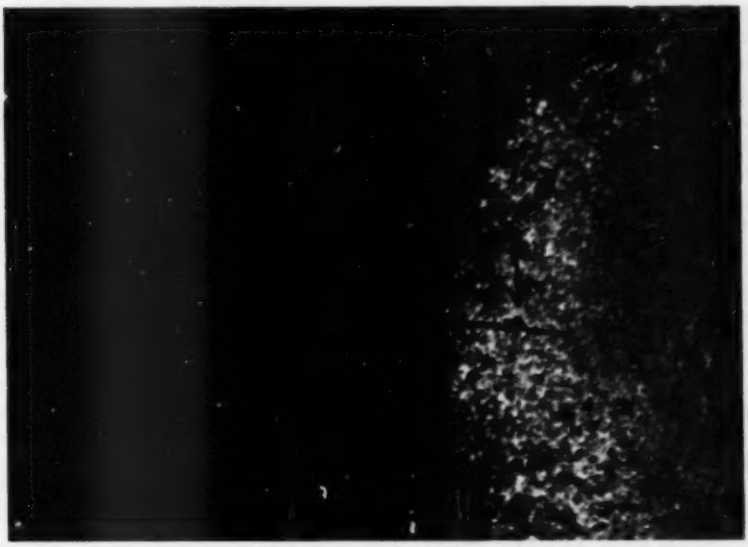

Fig. 4.—Chemotaxis (3+) of rabbit kidney (*right*) for rabbit leucocytes. The rim of the target (indicated by *bracket*) is densely infiltrated by leucocytes. Incubation 18 hours; × 115.

these days, gave relatively weak reactions, 1+ and 2+. Similar variations occurred on different days in tests on each of the other tissues. There were no technical variations to account for these differences. But the differences support the observations of Ketchel and Favour. We are convinced that many negative and weakly positive migrations of leucocytes to the targets result from some unknown factor in the plasmas from different animals.

In earlier reports † it was shown that sterile extracts of normal tissue, injected intracutaneously, not only caused capillary permeability but promptly led to the migration of the leucocytes into the area. No such migration followed similar injections of saline solution. Those chemotactic effects of tissue extracts in vivo are here supported by evidence of such effects in vitro.

SUMMARY

Tests for chemotaxis, performed by three methods, support the conclusions from our earlier report.[1]

Normal animal tissues, when allowed to autolyze briefly, contain water-soluble substances which attract leucocytes in vitro. It was shown previously that these extracts also attract leucocytes in vivo.

The substance of various animal tissues, when autolysis is minimized by freezing, attracts leucocytes in vitro. Our results support the evidence reported by others that some factor residing in the plasma of individuals causes variations in the migration of leucocytes in vitro.

The fact that normal tissues contain chemotactic substances supports the explanation that such substances released from damaged tissues act as the trigger mechanism which initiates acute inflammation.

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STRUCTURAL CHANGES IN INTRAPULMONARY ARTERIES EXPOSED TO SYSTEMIC PRESSURES FROM BIRTH

Report of a Case of Tetralogy of Fallot with a Widely Patent Ductus Arteriosus and an Anomalous Right Pulmonary Artery Arising from the Celiac Axis

CARLOS TEJADA VALENZUELA, M.D.
JULIO TORIELLO, M.D.
AND
WILBUR A. THOMAS, M.D.
BOSTON

STRUCTURAL changes in the intrapulmonary arteries in persons with congenital heart disease may play as important a part in the altered physiology as the anomalies in the heart and great vessels. In spite of their importance, these vessels have received relatively little attention in the literature.

A recent case presenting the basic anomalies of the tetralogy of Fallot with atresia of the pulmonary valve, plus a widely patent ductus arteriosus and an anomalous systemic artery arising from the celiac axis and supplying the lower lobe of the right lung, provided an unusual opportunity to study the intrapulmonary arteries under special conditions. We have made a comparative study of the intrapulmonary branches of the anomalous systemic artery with the regular pulmonary arteries receiving blood through the ductus arteriosus. In addition, we have studied the intrapulmonary arteries in a number of controls, including another case of tetralogy of Fallot with an atretic pulmonary valve but without a patent ductus arteriosus.

REPORT OF CASE

A male infant was admitted to the Massachusetts General Hospital for study at 2½ months of age because of recurrent cyanosis and retarded growth. He had been delivered at term by breech extraction and had been given oxygen for the first neonatal week. It was noted from birth that he became cyanotic on exertion.

His weight on admission was 5 lb. 2 oz. (2,325 gm.). The pulse was 104, and temperature was 99.2 F. The lips, feet, and hands were slightly cyanotic but became markedly cyanotic when he strained. A systolic murmur was heard best in the aortic area.

The erythrocyte count was 5,970,000 per cubic millimeter (normal value 4,500,000; Wintrobe[1]), and the hemoglobin was 15.9 gm. (normal value 12.2 gm.; Wintrobe). The electrocardiogram suggested right ventricular hypertrophy.

Conventional roentgenograms of the chest showed narrowing of the rib spaces on the right; congenital anomalies of the spine, and diminished vascularity of the left lung, with herniation of this lung across the midline (Fig. 1). The heart was enlarged and displaced to the right. There was evidence of pressure on the barium-filled esophagus posteriorly, consistent with an aberrant right subclavian artery (Fig. 2).

From the Department of Pathology and Radiology, Massachusetts General Hospital.

Kellogg Clinical Fellow in Pathology, Massachusetts General Hospital (Dr. Tejada Valenzuela), Kellogg Clinical Fellow in Radiology, Massachusetts General Hospital (Dr. Toriello), Clinical Fellow in Pathology, Massachusetts General Hospital (Dr. Thomas).

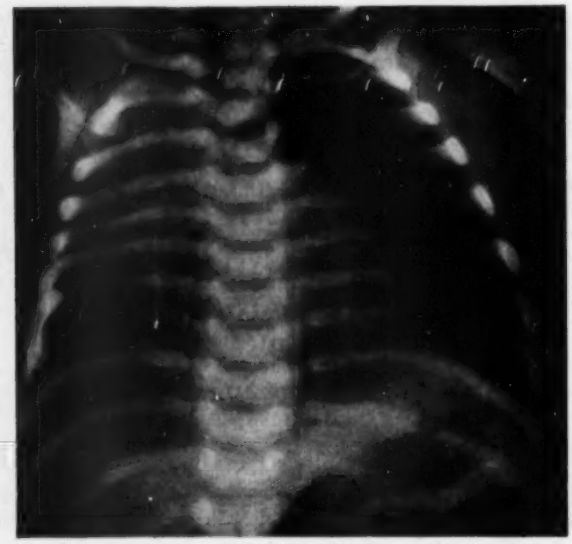

Fig. 1.—Anteroposterior conventional roentgenogram showing cardiac enlargement and displacement to the right, congenital anomalies of the spine and upper right ribs, and avascularity of the left lung.

Fig. 2.—Lateral view shows indentation of the barium-filled esophagus posteriorly, caused by aberrant right subclavian artery.

Because of the clinical and roentgenologic evidence of congenital heart disease, angiocardiographic studies were made to determine the exact nature of the anomalies. Eight cubic milliliters of 70% iodopyracet (Diodrast) was injected into the right external jugular vein, with prompt filling of the right ventricle and aorta and shortly thereafter filling of the left ventricle, suggesting an interventricular septal defect with dextroposition of the aorta. The left pulmonary artery was demonstrated, but it, as well as its branches, was small. The main pulmonary trunk and the right branch were not seen. A large anomalous artery was seen arising from the aorta below the diaphragm and extending obliquely cephalad into the right lung (Fig. 3). The

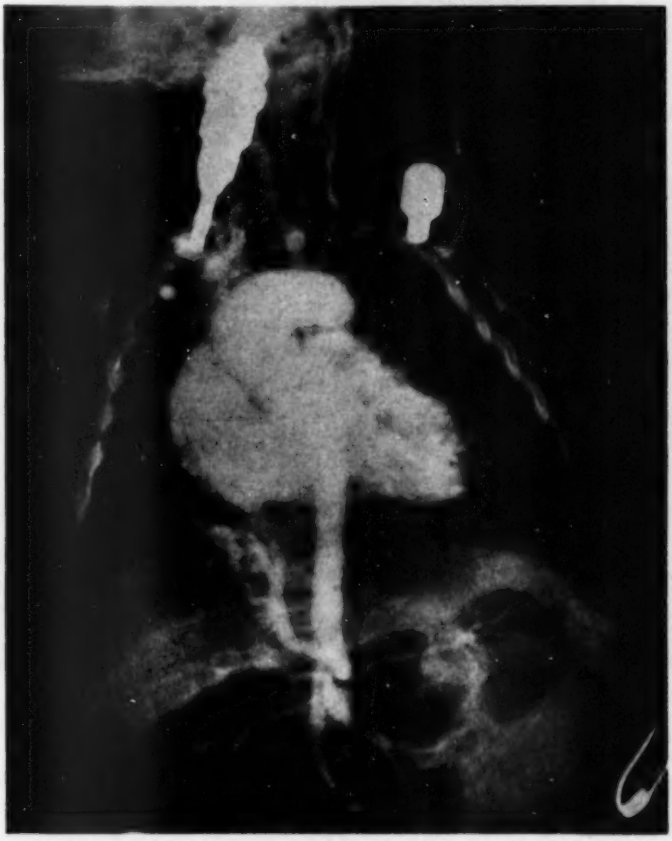

Fig. 3.—Angiocardiogram showing filling of the right auricle, right ventricle, aorta, and the large anomalous systemic artery supplying the lower lobe of the right lung. The small branches of the left pulmonary artery are filled, but the main trunk of the pulmonary artery was not visualized. There is some filling of the left ventricle.

roentgenologist interpreted this as consistent with tetralogy of Fallot and as sequestration of a large portion of the right lung, as manifested by the large anomalous pulmonary artery. No right pulmonary artery was seen.

The infant was kept in the hospital for the subsequent three and a half months but showed little change. His maximum weight was 6 lb. (2.7 kg.). He showed a slight elevation of temperature from time to time, but blood cultures were negative. By the time he was 6 months old, it had become apparent that survival was unlikely without some improvement in the oxygenation of blood.

At the age of 6 months an operation was performed, with transection of the left pulmonary artery and an end-to-end anastomosis to an anomalous right subclavian artery arising from the posterior aspect of the arch. A few minutes after the anastomosis was completed, the heart stopped, and independent contractions could not be reestablished.

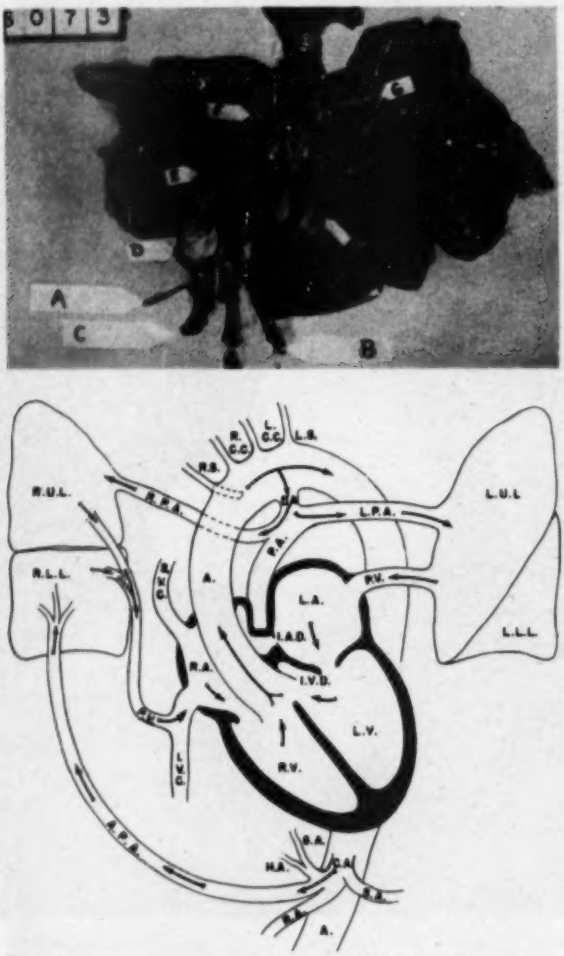

Fig. 4.—Above, anterior view of the heart and lungs. *A*, splenic artery; *B*, inferior vena cava; *C*, renal artery; *D*, anomalous artery to lower lobe of right lung; *E*, anomalous pulmonary vein connecting with the inferior vena cava; *F*, right pulmonary artery; *G*, anastomosis of left pulmonary artery to right subclavian artery. The arrow on the heart points to the interventricular septal defect. Below, diagrammatic presentation of circulatory relationships.

POSTMORTEM FINDINGS

The principal gross features are presented in Figure 4. The heart weighed 40 gm. and showed marked right ventricular hypertrophy. The right venticle measured 7 mm. in thickness; the left ventricle measured 5 mm. in thickness. There was an interventricular defect in the septum membranaceum measuring 7 mm. in diameter. The foramen ovale was probe-

patent but appeared functionally closed. There was dextroposition of the aorta, with the aortic valve overriding the interventricular defect. The aortic valve was bicuspid but otherwise negative. The right common carotid and right subclavian arteries arose separately. The latter originated from the posterior aspect of the aortic arch, and the former originated from the usual position of the innominate artery. The left common carotid and left subclavian arteries were normal in position. The ductus arteriosus arose a few millimeters beyond the level of the left subclavian artery and ended in the left pulmonary artery immediately beyond the bifurcation of the main pulmonary artery. It was cylindrical, widely patent, and approximately the same size as the left pulmonary artery. The atrioventricular valves, coronary system, and myocardium were normal. No gross abnormalities of the endocardium were noted, but on histological examination a small organized, partially calcified thrombus measuring 250 μ in diameter was found attached to a papillary muscle of the right ventricle (Fig. 8 below).

The lungs weighed together 37 gm. The main pulmonary artery originated in a blind pouch attached to the base of the heart a few millimeters to the left of the aortic valve but was

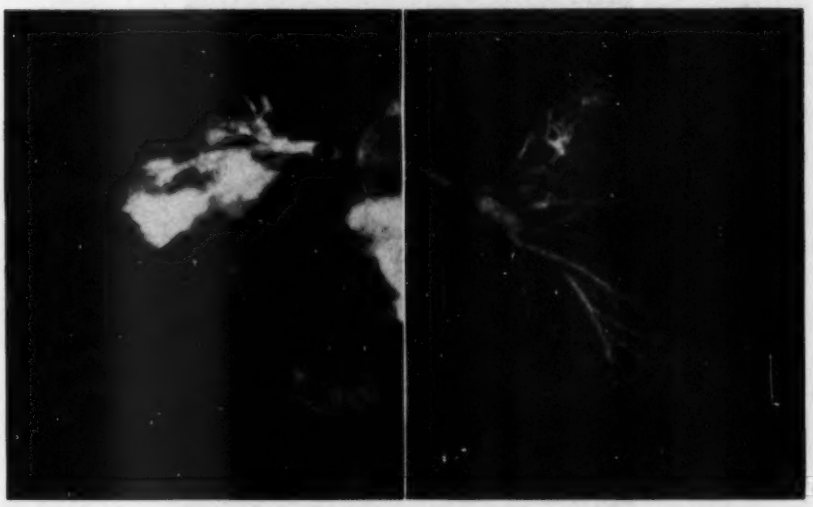

Fig. 5.—Left, postmortem roentgenogram of the right lung with the right pulmonary artery injected. Only the upper lobe is filled in this lung. Right, roentgenogram of the left lung with the left pulmonary artery injected. The entire pulmonary tree is filled in this lung.

otherwise unremarkable. The left pulmonary artery had been transected and anastomosed to the anomalous right subclavian artery. The latter was slightly smaller than the ductus arteriosus. The patency of the anastomosis and of the left pulmonary artery was demonstrated by injection of radiopaque material (Fig. 5 right). The right pulmonary artery was somewhat smaller than the left but was patent, as demonstrated by further injection of radiopaque material (Fig. 5 left). It supplied only the upper lobe of the right lung. The middle lobe was congenitally absent. The lower lobe of the right lung was supplied by a large anomalous artery branch from the celiac axis. It arose from a common trunk with the splenic artery and coursed cephalad through the retroperitoneal tissues and diaphragm. At this point it divided into two branches, each of which entered the inferomedial aspect of the lower lobe of the right lung. Injection of this vessel with radiopaque material showed that it supplied only the lower lobe of the right lung (Fig. 6). There was no atherosclerosis or grossly demonstrable thromboses of any of the pulmonary vessels.

The venous drainage of the left lung was through normally located pulmonary veins. No pulmonary veins entered the heart from the right lung. The entire venous drainage was through

a large anomalous vein which emptied into the inferior vena cava near the entry of the hepatic veins. Injection of the bronchial tree showed no abnormalities except absence of the middle lobe branch.

Histological examination of the lungs revealed striking changes throughout the arterial tree. These consisted for the most part of thickening of the muscular coats of the small pulmonary arteries (0.1 to 1 mm. external diameter, excluding adventitia) and retention of the fetal and neonatal muscular coat of the pulmonary arterioles (less than 0.1 mm.). There was no fibrous intimal thickening. These changes were present in all lobes, as could have been anticipated, since the lower lobe of the right lung received its blood supply directly from the abdominal aorta and the other lobes

Fig. 6.—Postmortem roentgenogram of the right lung, same as Figure 5 left but with the anomalous artery injected. Note that the lower lobe is now filled. The upper lobe remains filled from the previous injection shown in Figure 5 left.

received their blood supply from the thoracic aorta through the large ductus arteriosus. Comparison of these vessels with the normal vessels of corresponding size in the adrenals, kidneys, pancreas, and other organs supplied by the general circulation showed a nearly identical picture (Fig. 7 *A, B, C,* and *D*).

Another striking change was the presence of multiple organized recanalized emboli in many of the pulmonary arterioles and small arteries of the left lung (Fig. 8 above).

Histological examination of the ductus arteriosus showed a widely patent lumen, with no evidence of degeneration of the media, and only slight fibrous thickening of the intima.

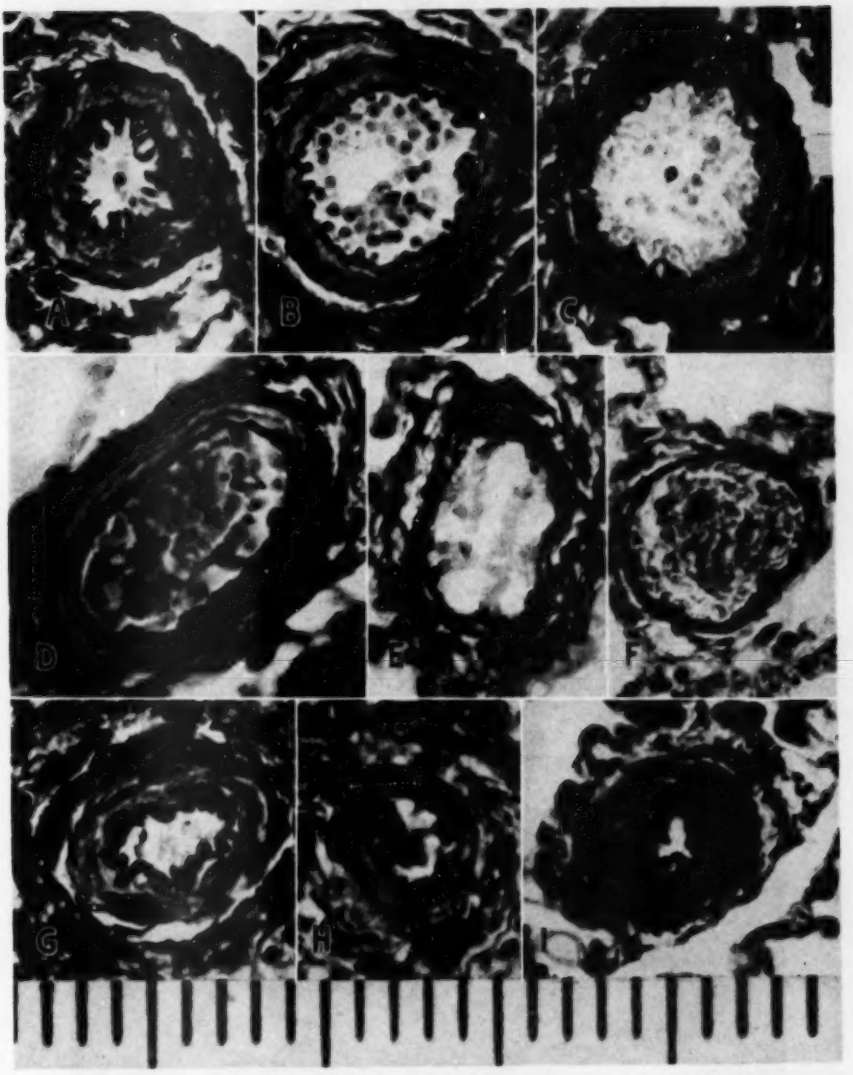

Fig. 7.—Sections of arterioles showing the thickness of the media in the case presented and of controls; stained for elastic tissue. All vessels are less than 100 μ in external diameter, excluding the adventitia. The exact size is indicated by the micrometer, which was photographed at exactly the same magnification as the vessels. Each small division represents 10 μ. Special care was taken to exclude vessels near bronchi, which could represent bronchial arterioles. The vessels pictured are as follows: Case presented, *A, B, C, D;* control lungs, *E, F, G, H, I. A,* lower lobe of right lung; *B,* upper lobe of right lung; *C,* left lung; *D,* adrenal gland; *E,* normal 6-month-old infant; *F,* 4-month-old infant with tetralogy of Fallot and with closed ductus arteriosus; *G,* 5-month-old infant with transposition of great vessels and interventricular septal defect; *H,* normal newborn infant; *I,* adult with mitral stenosis showing fibrous intimal thickening.

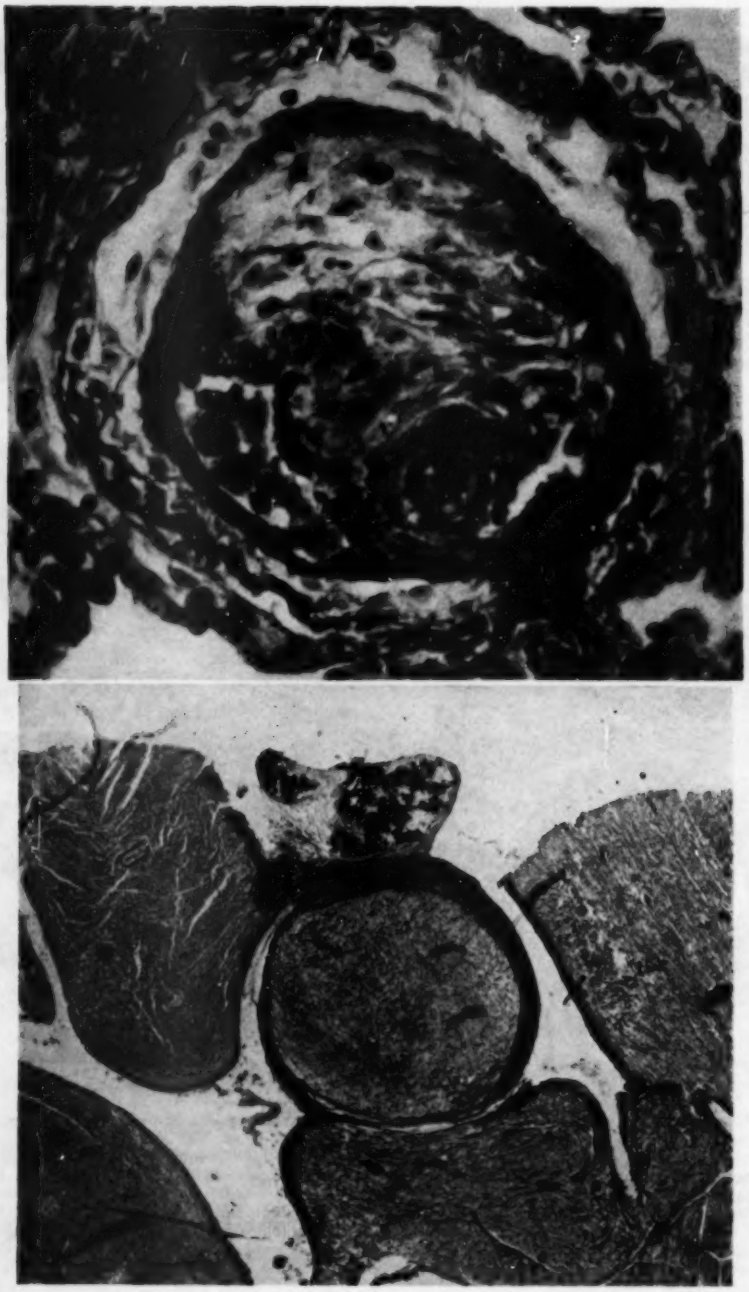

Fig. 8.—Above, organized embolus in small pulmonary artery; × 570. Below, organized mural thrombus attached to papillary muscle of right ventricle. Low magnification; × 50.

The only other findings of significance were agenesis of the left kidney and incomplete rotation of the colon.

CONTROL STUDIES

In order to more fully evaluate the changes in the pulmonary arteries, a comparative study was made with sections from 18 other cases. These included 1 newborn infant, 1 adult, 12 infants aged 1½ to 6 months with normal circulatory systems, and 3 persons with various types of cardiovascular abnormalities. Elastic tissue stains were done on all sections, and measurements of the arterioles and small

Comparative Measurements of Arterioles and Small Arteries

Age	Arterioles (Less Than 0.1 Mm.)				Small Arteries (From 0.1 to 1.0 Mm.)			
	Medial Thickening	Fibrous Intimal Thickening	External Diameter Wall, Ratio	Organized Thrombi	Medial Thickening	Fibrous Intimal Thickening	External Diameter Wall, Ratio	Organized Thrombi
Case Reported								
6 mo.*								
U. L. R. and left lung	+++	0	20%	+	+	0	15%	0
L. L. R.	++++	0	29%	0	+++	0	20%	0
Systemic arteries	++++	0	24%	0	+++	0	28%	0
Controls with Cardiovascular Abnormalities								
5 mo.†	++++	0	33%	0	++	0	21%	0
4½ mo.‡	0	0	7%	0	0	0	8%	0
Adult§	0	++++	30%	0	0	+++	20%	0
Controls with Normal Cardiovascular System								
2 days	+++	0	25%	0	..	..	....	0
1½ mo.	+	0	15%	0	0	0	8%	0
2 mo.	0 to +	0	9%	0	0	0	4%	0
3 mo.	0	0	9%	0	0	0	3%	0
3 mo.	0 to +	0	8%	0	0	0	4%	0
3 mo.	0 to +	0	8%	0	0	0	3%	0
4 mo.	0	0	6%	0	0	0	4%	0
4 mo.	0	0	7%	0	0	0	5%	0
4 mo.	0	0	7%	0	0	0	6%	0
5 mo.	0	0	9%	0	0	0	5%	0
5 mo.	0	0	7%	0	0	0	3%	0
5 mo.	0	0	5%	0	0	0	4%	0
6 mo.	0 to +	0	12%	0	0	0	4%	0
Adult	0	+	6%	0	0	++	4%	0

* U. L. R., upper lobe of right lung; L. L. R., lower lobe of right lung.
† Congenital heart disease; transposition of the great vessels with interventricular septal defect.
‡ Tetralogy of Fallot with closed ductus arteriosus.
§ Mitral stenosis (rheumatic heart disease).

arteries were made, using a technique similar to that described by Brenner.[2] The pulmonary arteries were divided into two groups: arterioles measuring less than 0.1 mm. and small arteries measuring from 0.1 to 1 mm. Special care was taken to exclude vessels near bronchi which could represent bronchial arterioles. Measurements were made of the media, the intima (if possible), the external diameter, and the thickness of the wall. The adventitia was excluded from all measurements. A percentage ratio was made between the external diameter and the thickness of the vessel wall. Numerous sections were studied in each case, and an average of the measurements was taken. The findings are recorded in the Table.

The small pulmonary arteries and arterioles in the controls from infants 1½ to 6 months of age had much thinner walls and larger lumens than those in the reported

case (Fig. 7 *E*). Study of the vessels in a 4½-month-old infant with tetralogy of Fallot, including atresia of the pulmonary valve and a closed ductus arteriosus, showed normal thin-walled vessels (Fig. 7 *F*), in striking contrast to those in the case under discussion. Sections from a 5-month-old infant with transposition of the great vessels and interventricular septal defect showed thick-walled muscular pulmonary arteries and arterioles similar to those in our case (Fig. 7 *G*). Section from a newborn infant showed the normal thick-walled vessels characteristic of fetal vasculature (Fig. 7 *H*).

COMMENT

In fetal life most of the blood from the right ventricle passes directly into the aorta through the ductus arteriosus, and relatively little passes through the lungs. This is made possible, in part, by increased resistance in the pulmonary arterial system, indicated morphologically by thick-walled small arteries and arterioles with correspondingly small lumens. At the end of fetal life the pathway through the ductus arteriosus normally closes, and the blood volume passing through the pulmonary tree increases tremendously. At the same time, the resistance in the pulmonary bed is decreased by dilatation of the small vessels, with resulting thinning of the muscular coats. Also, the pressure in the right ventricle and pulmonary artery becomes markedly decreased as compared with that in the left ventricle and aorta.*

If there is an anomaly of the circulatory system that results in persistent pressures in the pulmonary artery equivalent to those in the systemic circulation, the small arteries and arterioles will retain their fetal appearance and will resemble those in the tissues normally supplied by the general circulation.

This situation is clearly demonstrated in the current case. The intrapulmonary arteries in the lobes supplied by the regular pulmonary arteries are almost identical with those in the lobe supplied by the anomalous branch of the celiac axis and with those in the adrenals, kidneys, and other tissues supplied by the general circulation.

It is necessary to emphasize that this is a very unusual case † of tetralogy of Fallot and that these changes in the intrapulmonary vessels are exactly the opposite of those found in the usual case of tetralogy with a closed ductus arteriosus, as in Control 16. Moreover, most cases of uncomplicated patent ductus arteriosus do not show clinical or morphological evidence of pulmonary hypertension,[7] although such changes have been reported in some cases.[4]

The changes associated with pulmonary hypertension acquired later in life, as in mitral stenosis, are quite different. These consist largely of fibrous intimal thickening (Fig. 7 *I*), without striking changes in the muscular coats.

The pressure of the organized emboli provided an additional obstructive factor. Rich [8] studied a number of cases with the tetralogy of Fallot and found similar obstructive lesions in 90%. He interpreted these as thrombi which had formed locally in the pulmonary arteries as a result of slowing of the blood flow and the polycythemia. In our case it seems more likely that they are the result of embolization from the heart, since we found evidence of mural thrombosis in the right ventricle. In a recent study from this laboratory,[9] it was shown that in most cases of pulmonary vascular disease associated with cor pulmonale the cause was embolization rather

* References 3 and 4.

† References 5 and 6.

than localized thrombosis or arteriosclerosis. Moreover, the presence of the patent ductus arteriosus and the anomalous artery to the lower lobe of the right lung precluded any appreciable slowing of the blood flow, and there was no significant polycythemia.

SUMMARY

A case of complex congenital heart disease is presented which combines the basic anomalies of the tetralogy of Fallot with a widely patent ductus arteriosus and an anomalous branch of the celiac axis to the right lung.

A comparative study was made of the intrapulmonary arteries in the portion of lung supplied by the systemic artery and in the portion supplied by the regular pulmonary arteries through the ductus arteriosus. All showed thick muscular walls resembling those of vessels of corresponding size in other tissues supplied by the general circulation.

A number of control lungs were studied, including 12 from persons with normal circulatory systems in the same age group and 1 from a person with a similar type of tetralogy of Fallot but with a closed ductus arteriosus. Their thin-walled arteries contrasted sharply with those in the case presented.

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CALCAREOUS CHONDROPATHIES IN THE NEWBORN INFANT

WILLIAM C. YAKOVAC, M.D.
PHILADELPHIA

CONRADI,[1] in 1914, recorded the case of a newborn girl who showed, among other things, a calcareous type of chondropathy. The author concerned himself with the clinical and pathologic features of the skeletal changes and concluded that the calcium deposits signified a premature appearance of the secondary centers of ossification and that the total picture was compatible with a diagnosis of chondrodystrophia foetalis hypoplastica. Since this report, there have appeared in the world's literature to date some 40 additional case reports. In most of these, attention has been focused on the skeletal lesions.

In the reported cases, the patient is usually a newborn infant in whom there are manifest deformities of the axial and/or appendicular skeleton. Micromelia of one or more limbs, often accompanied by flexion deformities, is the single commonest skeletal defect encountered, although kyphoscoliosis and talipes, in any one of its variant forms, may also be found. Actually all, any one, or any combination of these deformities may be seen in any one patient. The same is true of saddle nose, large skull, and mental deficiency.

The x-ray picture is a striking one, in which multiple punctate calcific densities are seen within the substance of preosseous cartilage. This is often referred to as "stippling." The epiphyseal cartilages of the metatarsals and metacarpals are often spared, but the primary preosseous cartilages of the tarsi and carpi are quite frequently affected. The tracheal,[2] laryngeal, and hyoid cartilages * have been involved in a few instances.

Bilateral cataracts and/or some type of dermatosis (the skin has variously been described as being red, dry, thick, and scaly) have come to be associated with the skeletal disorder in approximately a quarter of all the cases. The cataracts in all but one instance have been congenital.[6] The simultaneous occurrence of disease in lens and cartilage suggests a relationship which is perhaps more than coincidental.

In 12 cases there are no details concerning survival or death. In 12 other cases, in which follow-up data are available, the patient, at the time the paper was written, was alive and at least a year old. In all but two instances, stippling of cartilage was the only finding, and with increasing age there was a gradual resorption of calcium deposits. In 1947, Fanconi [7] reported his findings in a patient whom Burckhardt [8]

Dr. Yakovac is a Trainee of the National Cancer Institute.

From the Laboratory of Surgical Pathology and the Penn Mutual Life Insurance Company Foundation for the Study of Neoplastic Disease, Hospital of the University of Pennsylvania.

Read before the Section on Clinical Pathology, Philadelphia County Medical Society, The Philadelphia Pathological Society, and Middle Eastern Region, College of American Pathologists, March 12, 1953.

* References 3, 4, and 5.

had reported in 1938. At 7 years of age, all stippling had completely disappeared, and growth of the long bones was not significantly delayed. Including the two cases to be reported, there were 19 deaths. The average age at death was 3½ months.

Serum calcium and phosphorus levels have been determined in 15 cases and were within normal limits in all but 2.† Serum alkaline phosphatase studies have been carried out in far fewer cases. Only once [9] has an abnormal level been reported. Consanguinity is reported in four cases.‡ Maternal health factors would appear to be noncontributory.§

In a few of the reports some manifestation of hematologic disorder is mentioned. The bleeding tendencies recorded in two of the cases below could well be ascribed to disordered liver function. Inasmuch as hematologic disorders figure prominently in my cases, the several other reports in which it has been noted are cited briefly.

Conradi's patient was a female infant who died at the age of 5 weeks.[1] The findings at birth included a hematoma of the scalp, bilateral cataracts, thick scaly skin, shortening of the right femur, and enlargement of the liver. There was no jaundice. X-rays showed stippling of most of the cartilages of the skeleton. Terminally, bloody stools developed. Pathologic descriptions were limited to bones and cartilage.

Coughlin and co-workers [27] described the case of a girl who died at the age of 3 months. There was symmetrical shortening of the femora and humeri, again in association with x-ray evidence of stippling. At autopsy, in addition to the skeletal changes, the following histologic findings were noted: hemorrhage into the alveoli of the lung, an increased amount of blood pigment in the spleen, and many megakaryocytes in the bone marrow.

In deLange and Janssen's case report [28] the patient was a girl who died at the age of 9 weeks. The forearms and legs were shortened. X-rays taken at 7 weeks of age revealed stippling of the pelvis, trochanters, femora, patellae, and calcanei. There were also facial asymmetry, in which the mouth was drawn to the left (paralysis?), and pes varus. Six weeks after birth, jaundice and hepatomegaly were noted. Terminally, hematomas, followed by necrosis, appeared over the cheek and back. The red blood cell count reached a low of 3,800,000. The jaundice deepened, and the stools became bloody. The patient's father was Rh positive, and the mother, whose first child was stillborn, was Rh negative, but a high titer of agglutinins could not be demonstrated in the infant. At autopsy, microcysts, an increase of interstitial tissue, round cell infiltration, and a "malformation of the tubular system" were reported in the kidney. The liver showed hypogenesis of the bile ducts.

Blatt and co-workers [9] presented data on a prematurely born boy who died at the age of 9 weeks. All limbs were symmetrically shortened. Stippling was widespread, and the condition was referred to as "epiphyseal dysgenesis." The child was labelled a cretin. The red blood cell count was 3,000,000. Serum alkaline phosphatase was markedly elevated on a single determination. A 24-hour urine specimen disclosed a decreased creatine-increased creatinine output. Serum cholesterol levels were consistently low normal. At autopsy, the statement was made that

† References 5 and 6.

‡ References 10, 11, 12, and 13.

§ References 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, and 26.

numerous megakaryocytes were found in the bone marrow. Whether this finding and the similar one by Coughlin and co-workers imply an abnormally large number or not is unknown.

Gørtz's case,[3] cited by Mosekilde, was that in a boy who died at 3 weeks of age. No autopsy was performed, but clinically there were bilateral congenital cataracts, shortening of the humeri and femora, stippling of preosseous and laryngeal cartilages, and hematuria, bleeding from the umbilicus, and a hematoma of the scalp.

Swoboda's patient [29] was a boy in whom were discovered three days after birth a marked anemia, slight hemorrhagic diathesis, and icterus neonatorum. The etiology of the anemia was not clear. X-ray showed stippling of one calcaneus only. The skeleton otherwise was not remarkable. By the age of 4 years the stippling had completely disappeared. (At the age of 2 years a slight angular lumbodorsal kyphosis was observed.)

Including my two cases, hematologic disorders are found in 16% of all reported cases and are associated with a mortality of 85%.

Many descriptive names have been given to this condition in the past 39 years, all focusing attention on the skeletal changes and directing it away from the total picture.

Hünermann,[30] in 1931, designed a term most authors since have adopted, "chondrodystrophia calcificans congenita." Lang and Priesel [31] and Kwerch [32] prefer to substitute "dysplasia" for "dystrophia." Fanconi [7] employs the term "chondrodystrophia calcarea." "Punctate epiphyseal dysplasia" was suggested by Fairbank ‖ and later Latinized by him to "dysplasia epiphysialis punctata." Cocchi,[25] in referring to the disorder as "chondroangiopathia calcarea seu punctata," emphasized the pertinent microscopic characteristics of the cartilaginous lesions. Hilliard [26] refers to his case as one of "chondro-osseous dystrophy with punctate epiphyseal dysplasia," and Gørtz [3] refers to hers as "chondrodystrophia hypoplastica calcinosa." Lightwood's term, "stippled epiphyses," refers to the x-ray picture.[12] Strictly speaking, it is an incorrect term, for an epiphysis, by definition, is already bone which has formed in cartilage by the process of endochondral ossification and represents a secondary center of ossification. Another disadvantage of Lightwood's term is that it does not take into account the fact that stippling can occur in the small preosseous cartilages, such as those of the carpi and tarsi, in which ossification begins in only one center and in which there is no participation in the growth process by periosteum (perichondrium).

It is the purpose of this paper to present the clinical and pathologic findings in two newborn infants who show, in common, skeletal defects associated with calcium deposits in the primary and secondary preosseous cartilages.¶ However, the gross, microscopic, and x-ray features of the cartilaginous lesions in the two cases are distinctly dissimilar, as are the underlying disease processes. These findings suggest that some revision is necessary in the current concepts which hold that calcareous chondropathies in the newborn infant represent an entity sui generis or that they represent an abortive form of some other well-defined disease of cartilage, notably achondroplasia (chondrodystrophia foetalis).

‖ References 33 and 34.

¶ The terms primary and secondary refer to centers of ossification.

REPORT OF CASES

CASE 1 (H. U. P. Autopsy No. 52-138).—A Negro boy was stillborn June 5, 1952, after an essentially uneventful gestation.

Maternal History.—The mother, M. E., a Negro, aged 27, was found to have a positive serologic test for syphilis for the first time in 1944. Her husband also had syphilis. A course of heavy metal therapy was instituted and continued for three years. In 1949 a repeat serologic test for syphilis was reported as positive. She was then given 20 daily injections of penicillin and subsequent serologic examinations have remained negative. In July, 1951, a diagnosis of rheumatoid arthritis was made. Treatment with gold, 17-hydroxycorticosterone (Compound F), liver, iron, multiple vitamins, and yeast was begun. (At the time of this pregnancy the patient was separated from her husband and had not remarried. The alleged father of the child was not a blood relative of the mother. The status of his health could not be determined.)

In September, 1952, serum calcium and phosphorus, cholesterol, and alkaline phosphatase determinations were made. All results were reported as being within normal limits. A repeat serologic test for syphilis was negative.

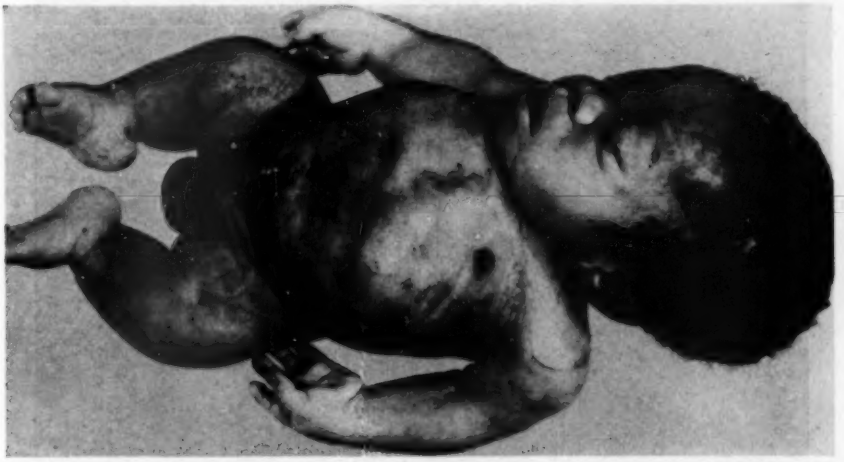

Fig. 1 (Case 1).—Stillborn infant with shortened humerus, saddle nose, short neck, bell-shaped thorax, and pes varus.

The patient volunteered the information that she took acetophenetidin (Anacin, a proprietary compound) for her arthritis (6 to 12 tablets per day from 1948 to July, 1952).

Autopsy.—Gross examination showed weight 2,250 gm., height 34 cm. Skeletal changes were striking and included marked dolichocephaly; saddle nose; short neck; bell-shaped thorax, with striking flaring of the lower ribs; shortening of the upper arms and thighs, and an extreme degree of pes varus (Fig. 1).

Because of these findings, x-rays of the whole body were obtained prior to dissection. Discrete punctate densities were seen to affect the preosseous cartilages of most of the skeleton. Bones formed in membrane such as the calvarium, facial bones, and clavicle were not involved. Also exempt were those bones at the base of the skull which are preformed in cartilage, the phalanges of both hands and feet, the metacarpals and metatarsals, the proximal end of the radius, the distal end of the ulna, and the patella. The humeri, femora, and tibiae were shortened. Distinct secondary centers of ossification were not recognizable as such in the calcanei and distal ends of the femora (Fig. 2).

The cartilaginous defects were identical in all of the involved bones. The external contour of the cartilaginous epiphyses were not remarkable, but on section the central regions lacked the blue milky opalescence of normal cartilage. The tissue was softened and fibrous, and creamy

yellow gritty calcific particles and plaque-like scales were embedded in it. The margins of this malacic cartilage, where it merged with relatively uninvolved tissue, were tinted a light yellow-brown. The line at the osteochondral junction was generally even.

Other gross findings of note included a shallow posterior cranial fossa, large hemorrhages about the midthoracic portion of the descending aorta and beneath the liver capsule, and smaller hemorrhages beneath the visceral pleura and in the right lobe of the cerebellum.

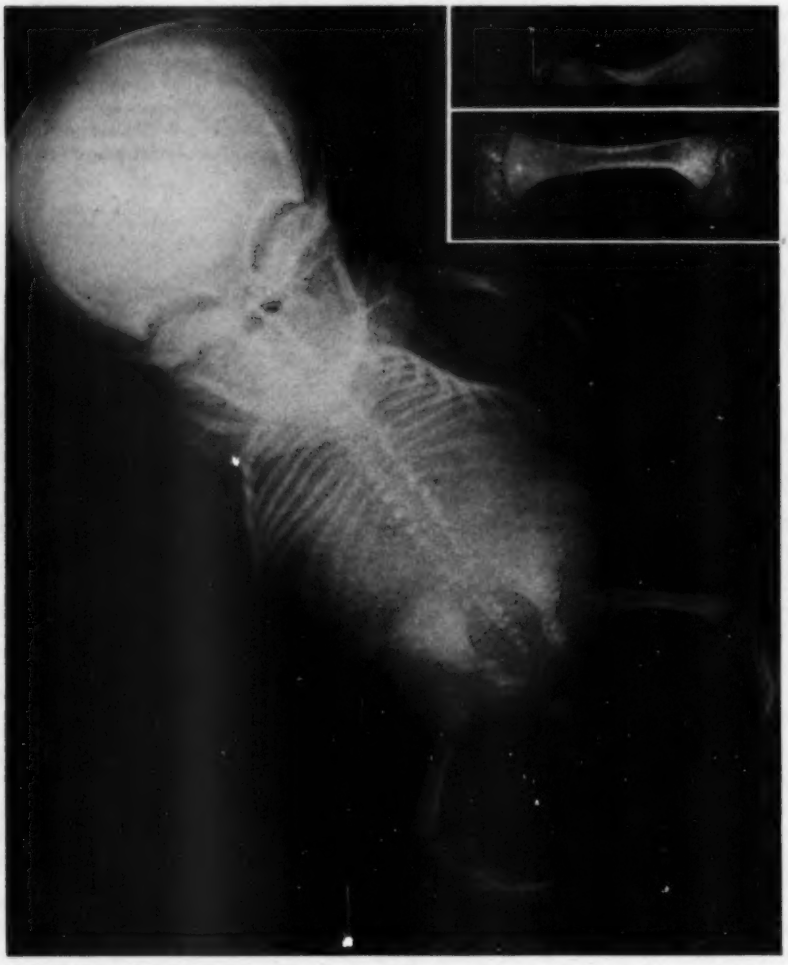

Fig. 2 (Case 1).—Roentgenogram showing diffuse stippling of primary and secondary preosseus cartilages, with marked involvement of spinal column. Femora and tibiae also shortened. Inset, roentgenogram of long bones individually dissected showing distribution and nature of calcific particles within cartilage. Contour of cartilage heads is not distorted.

The organ weights were as follows: thymus 9 gm., heart 15 gm., lungs 13.5 gm. left and 15.0 gm. right, liver 86 gm., spleen 7 gm., kidneys 11 gm. left and 10 gm. right.

Dark-field examination of the cord blood was negative for Treponema pallidum.

Microscopic Examination.—Skeleton: The carpus, tarsus, radius, ulna, tibia, femur, humerus, patella, spinal column, and calvarium were examined histologically. There was focal dissolution of ground substance in the preosseous cartilages of all bones. In these degenerative areas there was marked proliferation of the cartilage canals. The connective tissue elements of the canals were condensed into dense eosinophilic bands at the margins of the lesion. The vessels were engorged, and here and there small hemorrhages were seen. In addition, there were areas of calcification of irregular shape and size situated in the zones of resting cartilage. The calcified cartilage was being eroded from without by thin-walled vascular channels. Multinucleated osteoclasts were prominent (Fig. 3). In a few instances some osteoblastic activity was noted.

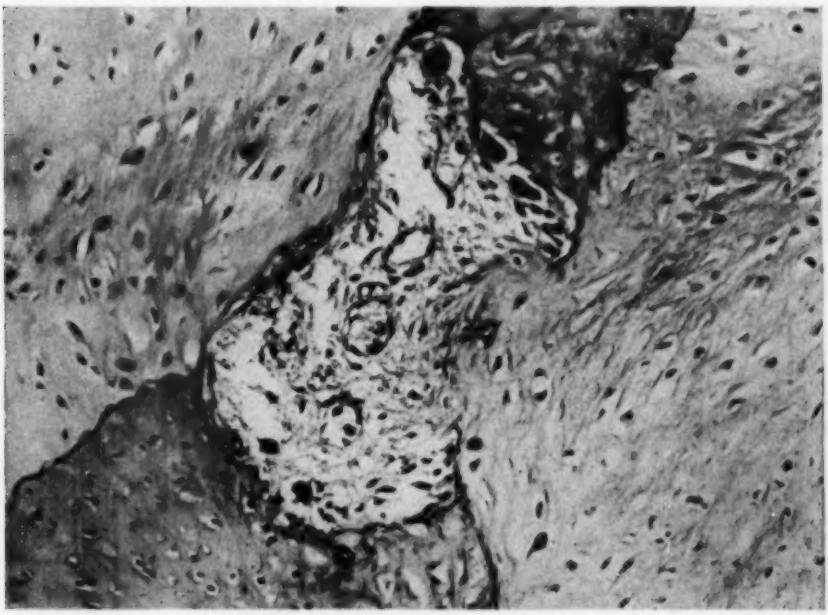

Fig. 3 (Case 1).—Erosion of calcified cartilage by thin-walled vascular channels. Note alteration of adjacent ground substance. Distal end of femur; × 150.

On the whole the zones of proliferating and maturing cartilage were orderly in arrangement, and osteoblastic activity appeared to be unhampered. Occasionally, however, in focal distribution, the characteristic column formation of both the proliferating and the maturing cartilage cells was disturbed (Fig. 4*A*). This interference in the actively growing portion of the cartilage apparently affected the constant supply of maturing cells required for maintaining bone growth at the provisional zone of calcification. At these sites, therefore, the horizontal linear pattern of the osteochondral junction was uneven.

A fibrous band, apparently arising from the periosteal-perichondral junction, was noted to traverse the active growing zone of the distal end of the tibia. This finding, a "periosteal streak," has been considered as pathognomonic of chondrodystrophia foetalis (achondroplasia) (Fig. 4*B*).

The matrix about the cartilage cells in the hypertrophic zone contained a peculiar yellow-brown granular pigment. Its exact nature is not known, but it did not contain iron.

An incarcerated island of hyaline cartilage undergoing ossification was seen in the medullary cavity of the middiaphysis of the right tibia. Another smaller bit of hyaline cartilage was situated in the cambium layer of the same bone, also in midposition.

The hemopoietic elements of the bone marrow appeared active. The cell population was essentially normal, except for an absolute increase in the number of megakaryocytes, most of which showed swelling, vacuolization, loss of granulation of the cytoplasm, and nuclear distortion of varying degree. While lobulated forms

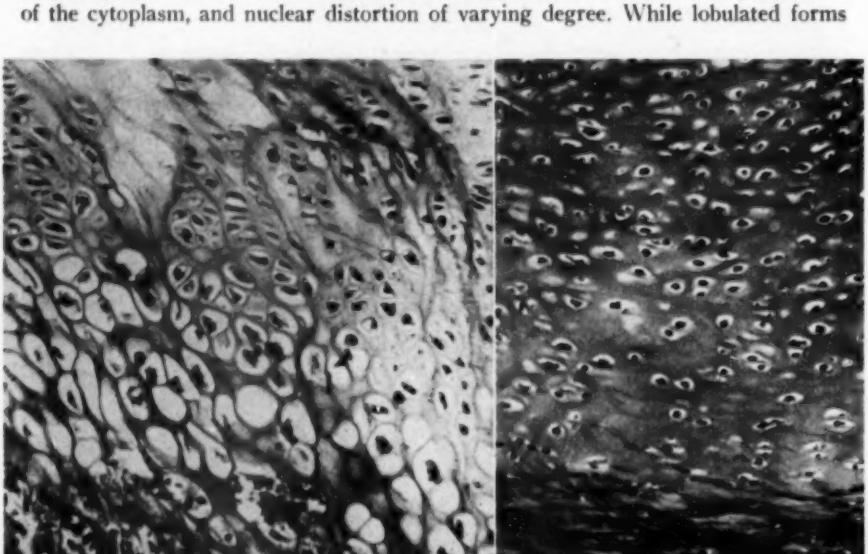

Fig. 4 (Case 1).—*A*, column formation in proliferating and maturing zones is distorted. Invasion by capillaries from the marrow is unhampered. Proximal end of femur; × 108. *B*, so-called "periosteal streak," in the distal end of tibia; × 200.

were occasionally seen, the nucleus, in most instances, was represented by a single eccentrically situated spherical hyperchromatic body. Anuclear masses of finely reticulated pale basophilic-staining cytoplasm were numerous (Fig. 5*A*).

Heart: There were several foci of altered collagen in the leaflet of the mitral valve. The dense blue granular appearance of these foci suggested calcification. However, calcium could not be demonstrated by the von Kossa method.

Blood Vessels: In the media of the ascending aorta were several areas where the collagen fibers had the same altered appearance as those in the mitral valve. The von Kossa reaction was again negative. A large hemorrhage surrounded the wall of the descending aorta, lying between it and the parietal pleura. Many arterioles in all the organs examined contained subintimal deposits of granular strongly

basophilic material (Fig. 5*B*). These, too, failed to give a positive result with the von Kossa reaction. That they did not contain calcium was further substantiated by the fact that their staining reaction remained unaltered when frozen sections of a formalin-fixed tissue were placed in 0.1 N HCl. Specific stains for iron, however, revealed markedly positive results.

Myriad platelets were found in the alveolar spaces of the lungs and within the vascular lumina in all the organs. These findings suggest that the subintimal masses

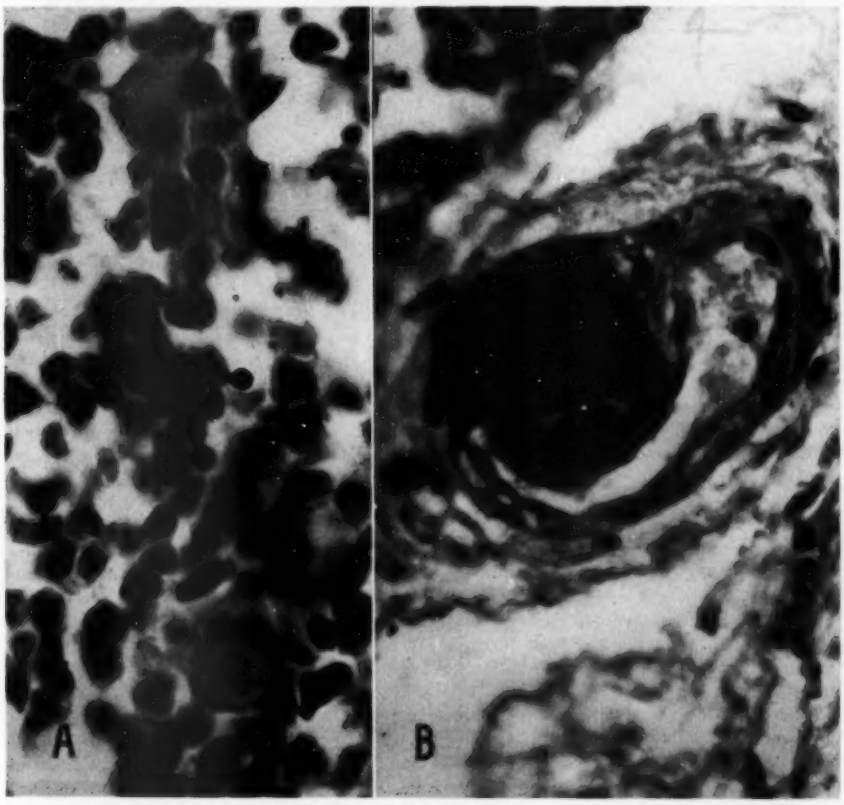

Fig. 5 (Case 1).—*A*, abnormal megakaryocytes in uppermost field show nuclear distortion and swelling of cytoplasm; × 620. *B*, subintimal granular mass in arteriole of lung. Lumen is filled with platelets; × 450.

were platelet thrombi. None of the thrombi were organized. Megakaryocytes in the circulating blood, especially in the capillaries of the lung, where others have reported seeing them in this condition, were not observed in this case. Platelets were conspicuously absent in the sinusoids of the liver, and nowhere in any of the multiple sections of this organ were formed thrombi seen.

Lungs: The bronchial cartilages were normal. Keratin strands were present in unexpanded alveoli.

Liver: There was evidence of minimal extramedullary hemopoiesis.

Spleen: Large amounts of hemosiderin pigment were present in the spleen. No evidence of blood formation was observed.

Brain: The right lobe of the cerebellum contained multiple small hemorrhagic necroses.

There was no histologic evidence of cataract. The parathyroid glands were normal. Other organs which were examined histologically and in which no lesions were found included the pancreas, kidney, bladder, prostate, testes, gastrointestinal tract, adrenal gland, thyroid, thymus, and skin.

CASE 2.—J. L. S., a 6-week-old white girl, was admitted to the Children's Hospital on May 3, 1952, and died on July 25. On admission, the chief symptoms were jaundice, bone condition, and rash, all noted since shortly after birth.

The baby's birth weight was 5 lb. 2 oz. (2.3 kg.). She was kept in an incubator with oxygen for three days. On the fourth day it was noted that the child was not moving her shoulders, although she did move her elbows and legs. X-rays taken at that time were diagnosed as "chondrodystrophia calcificans congenita." The child ate poorly, her weight never exceeding that at birth. Jaundice had persisted since the third day, and the patient had a polycythemia, with a hemoglobin persistently around 140%. The baby also had a rash on her face intermittently for periods of several days.

The family history revealed only that a paternal grandparent had diabetes. There was no other known familial disease.

On admission, the child was afebrile; her weight was 5 lb. 2 oz., length 18 in. (45.72 cm.), head 12⅞ in. (33.00 cm.), chest 11⅝ in. (29.21 cm.). She appeared well nourished and of reasonable hydration. There was a reddened papular rash over the face and lower one-third of the scalp and back of the neck. The anterior fontanelle was open and soft; the posterior fontanelle was closed. There was questionable icterus of the sclerae, and the pupils did not seem to react to light. The child's upper arms appeared shortened, and there was limitation of shoulder motion in elevation above 90 degrees. Abduction was limited at the hip. On neurologic examination, no tendon reflexes could be elicited. The Babinski reflex was not elicited, and there was a suggestion of a left facial paralysis. The child was irritable but responded normally to affection. Physical examination was otherwise negative.

Initial laboratory studies revealed a trace of protein in a bile-positive urine. Other studies showed hemoglobin 14 gm.; 3,500,000 red blood cells; 11,600 white blood cells; 24% neutrophiles; 67% lymphocytes; 3% monocytes; 6% eosinophiles; platelet count 500,000; prothrombin time 100%; bleeding time two minutes; coagulation time four minutes. Red blood cell fragility studies revealed an initial hemolysis at 0.42% salt solution and complete hemolysis in 0.3% salt solution. Coombs test was negative. Serologic test for syphilis was negative. Results of old tuberculin test (dilution 1:1,000) were negative. Blood calcium 10 mg. per 100 cc.; phosphorus 4.9 mg. per 100 cc.; total bilirubin 3, one minute direct 1.4 mg. Thymol turbidity 0.5 units. Total protein 7 gm. per 100 cc., N.P.N. 30 mg. per 100 cc.

The initial x-ray studies were reported as follows: "The secondary centers of ossification of both shoulders, both elbows, both wrists, both knees and both hips all show an abnormal calcified stippling. Both lower extremities and both humeri are short compared with the length of the trunk. The width of these bones appears normal" (Fig. 6*A*). Chest and skull x-rays showed nothing abnormal. Repeat x-rays of the upper and lower extremities six weeks later showed no significant change. An abdominal film demonstrated no evidence of renal calcification.

Slit lamp examination by the consultant ophthalmologist revealed congenital cataracts bilaterally. In position they were subcapsular, posterior, and densest temporally. The "Y" sutures were clearly seen in the two eyes posterior to the lens.

Further history from the mother at that time revealed that she had received many injections of various kinds throughout the pregnancy and also that a hound dog kept outside her house had had "yellow jaundice" for two months. After admission, the child continued to eat poorly, and at times it was necessary to feed her by tube or even parenterally. Thus, her caloric intake was kept at about 90 Cal. per pound per day. Nevertheless, her weight gradually fell from 5 lb. 2 oz. to 4 lb. 7 oz. (1.8 kg.) at the time before her death. The slight jaundice present on admin-

sion gradually disappeared completely. The child was afebrile throughout her illness. On June 7, 1952, oral administration of progesterone, 5 mg. daily, was begun in hopes of stimulating appetite and possibly of decalcifying abnormal deposits. Four days later the administration of progesterone was stopped because of increasing irritability of the child and because there was no noticeable beneficial effect. A few days later she was given another course of progesterone,

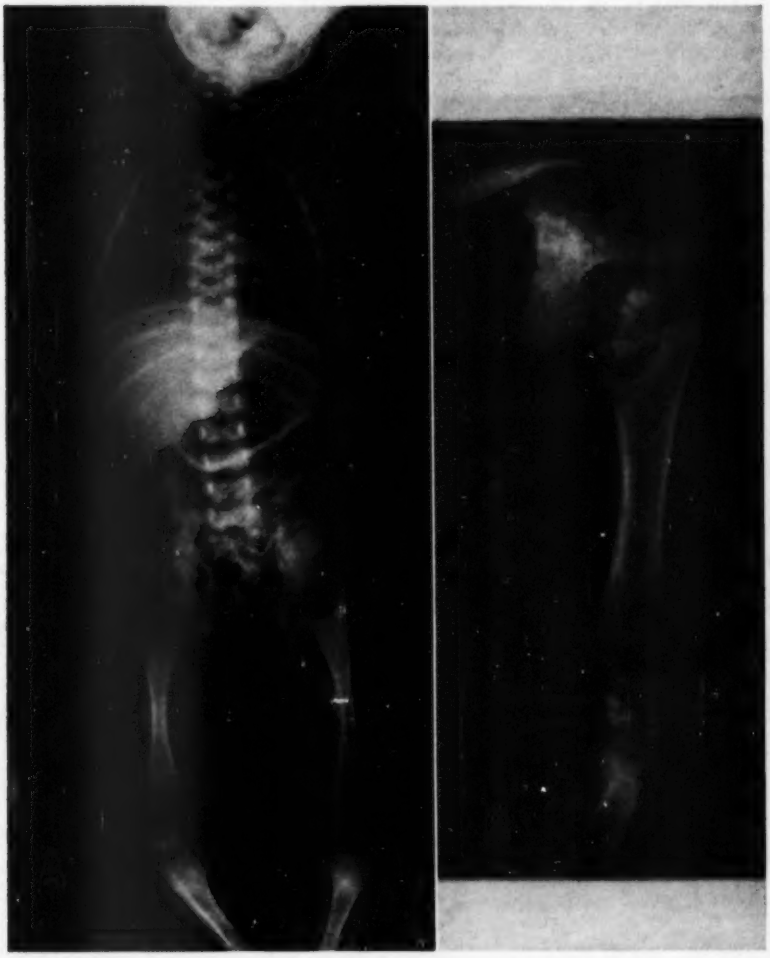

Fig. 6 (Case 2).—*A*, calcific stippling in preosseous cartilages of long bones, pelvis, and shoulder girdle. Vertebral column is spared. *B*, roentgenogram of left humerus after dissection post mortem. Calcific deposits are coarse and confluent. Cartilage heads are swollen and distorted.

2.5 mg. daily for five days, without benefit. On June 17, 1952, she was given corticotropin (ACTH), 5 mg. every eight hours. Administration of this drug was stopped after seven days, without evidence of any clinical improvement.

Follow-up laboratory work during hospitalization revealed a hemoglobin range from 10 to 14 gm. prior to transfusion on May 16, 1952; thereafter it ranged from 13.6 to 16 gm. The red

blood cell count ranged from 2,700,000 to 3,500,000 prior to transfusion and was about 4,400,000 thereafter. The white blood cell count ranged from 10,000 to 18,000. There was a predominance of lymphocytes in the differential count at the time of admission. This gradually changed until there was an equal number of neutrophiles and lymphocytes, with 2 to 3% monocytes and 1 to 6% eosinophiles. An eosinophile count on June 18, 1952 (after beginning administration of corticotropin), revealed only 44 cu. mm. of blood. Carbon dioxide and chlorides were normal on May 31, 1952, but the chlorides terminally rose to 112 mEq. per liter. At that time N.P.N. was 29 mg. per 100 cc., and alkaline phosphatase was 5 Bodansky units. A Sulkowitch test done on the urine July 4, 1952, gave a 3+ result; whereas a urinalysis done three days later showed a slight trace of protein and 10 to 12 granular casts. These granular casts had also been reported in one previous urinalysis approximately three weeks before. Qualitative stool examination on July 23, 1952, run on a 24-hour sample, showed total nitrogen 493 mg., N.P.N. 433 mg., protein 423 mg., total volume 204 cc. in 24 hours.

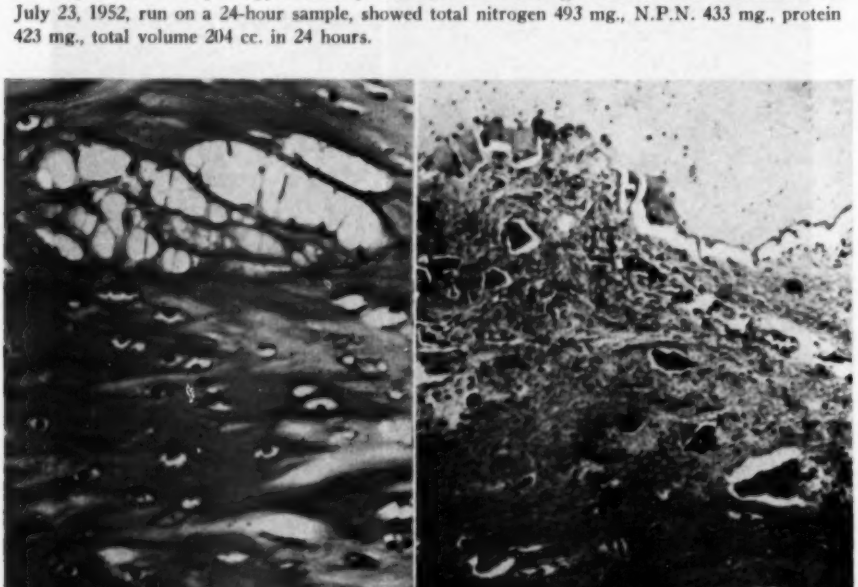

Fig. 7 (Case 2).—*A*, edema of cartilage, smudginess of ground substance, and coalescence of lacunae to form cleft-like spaces. Distal end femur; × 200. *B*, complete necrosis of cartilage is seen in the lowermost zone. Separating this from the uppermost zone of normal-appearing cartilage is an intermediate band of vascular connective tissue bearing osteoblasts, osteoclasts, and islands of osteoid matrix. Proximal end of humerus; × 88.

During the last few days of life the child failed to take fluids and required frequent aspiration and oxygen. Her condition deteriorated progressively and she died on July 25, 1952.

The clinical diagnoses were "chondrodystrophia calcificans congenita," malnutrition, and jaundice of undetermined origin.

Autopsy.—Autopsy was performed by Dr. George Ward. The child's head was larger than normal, and frontal bossing was prominent. The anterior fontanelle was open but not bulging. The lenses of the eyes showed opacification. The elbow and knee joints were greatly enlarged, but the skeleton showed no other obvious abnormalities.

Incisions revealed a decrease in the amount of subcutaneous and mesenteric fat and a thickening of the calvarium.

Skeleton: All the larger epiphyseal cartilages of the long bones showed great enlargement, and the contours were distorted (Fig. 5*B*). Section revealed many large confluent areas of softening in which particles of a gritty substance, presumably calcific, were embedded. The cancellous bone of the marrow cavity offered little resistance to cutting.

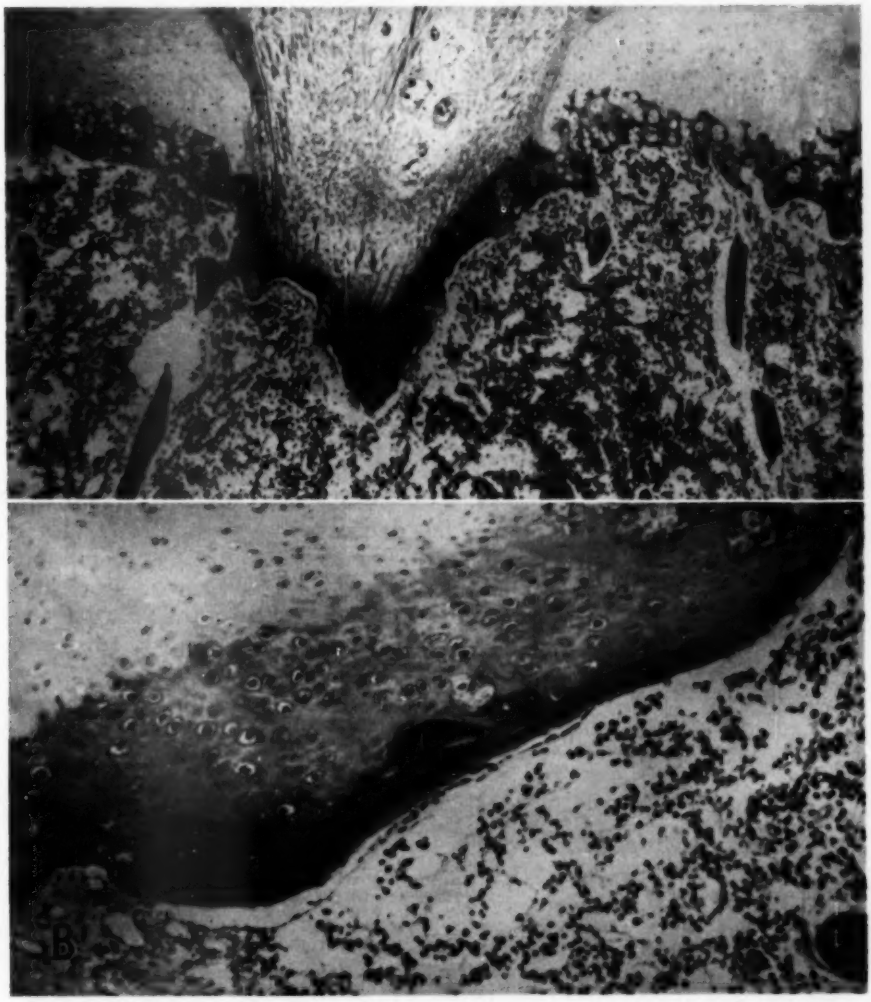

Fig. 8 (Case 2).—*A*, section from proximal end of femur at osteochondral junction showing sluggish osteogenetic activity. Note band of necrotic cartilage which is poorly calcified and sparse trabeculation of marrow cavity. Arrow-shaped projection in center suggests membranous bone formation; × 40. *B*, shelf of osteoid matrix apposed directly on a broad band of necrotic cartilage. Proximal end of femur; × 108.

Lungs: The lungs were dark red, spongy, and wet. On compression frothy sanguinous fluid admixed with purulent material could be expressed from the cut surface. Areas of consolidation and hemorrhage were present. The trachea and bronchi did not appear remarkable.

Brain: The brain showed definite abnormally widened gyri in the region of the posterior frontal lobe, particularly in the region of the motor strip. This was associated with microgyria at the tip of the frontal pole and a very wide Sylvian fissure, through which the island of Reil could be seen without retracting the overlying brain.

Organ weights were as follows: heart 16 gm., lungs 63 gm. combined, liver 72 gm., spleen 8 gm., kidneys 12 gm. combined, adrenals 2 gm. combined.

Microscopic Examination.—Skeleton: The humerus, femur, patella, and spinal column were examined histologically. The cartilages presented extremely variable and complex changes. Most prominent was the focal loss of the homogeneous glossy pale basophilic characteristics of normal hyaline cartilage. The ground sub-

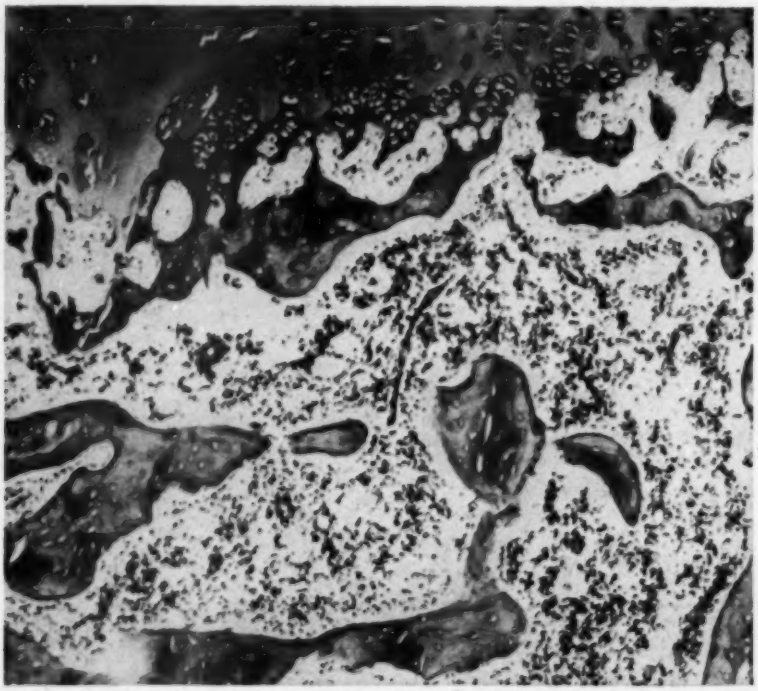

Fig. 9 (Case 2).—Trabeculae irregularly disposed in relation to the longitudinal axis of the left humerus; × 88.

stance appeared edematous and had irregular tinctorial qualities in the nature of dense blue streakiness or smudginess. The lacunae were swollen and distorted, and some coalesced to form large clefts (Fig. 7*A*). In other areas there was complete dissolution of the ground substance, and all that remained was the eosinophilic-staining collagenous framework enmeshing cell-poor lacunae. Proliferation and engorgement of the vascular cartilage canals were conspicuous. Again in other areas this process appeared to have gone on to complete necrosis, where centrally there was loss of all cellular detail. A wall of fibrous tissue surrounded the necrotic debris, and fibroblasts were beginning to grow in. At the periphery there was sharp delineation between the diseased cartilage and the normal cartilage surrounding it. The

margins here were peppered with small irregularly shaped islands of calcified cartilage, about which some osteoid was being laid down. In the intermediate zones between the periphery and the area of central necrosis was vascularized connective tissue bearing many osteoblasts and osteoclasts and islands of osteoid matrix. Marrow cavities were not present (Fig. 7*B*).

The disturbance of cartilage apparently affected all zones, with total distortion of the actively growing zones. Maturation of cartilage cells had virtually ceased; calcification of cartilage matrix was slight, and the process of endochondral ossification was sluggish at best. In those instances in which the complete dissolution of ground substance had been extended to involve the growing zones, the process of ossification mimicked that of membranous bone formation. Where, however, the cartilage had become necrotic, a shelf of osteoid matrix was apposed directly on its diaphyseal aspect (Fig. 8 *A* and *B*).

The bony trabeculae formed at the osteochondral junction were irregularly aligned with relation to the longitudinal axis of the bone (Fig. 9). The basophilic core of calcified cartilage was inconspicuous. In the diaphysis there were no trabeculae as such. All that remained were insignificant sparse bits of osteoid matrix widely separated by marrow tissue. Also in the diaphysis were large empty intercommunicating cyst-like spaces, lined in places by a thin granular eosinophilic membrane. Generally, periosteal bone was unaffected, although it was extremely thin in isolated areas. Hemopoiesis appeared normal.

Liver: The hepatic sinusoids were distended and in many instances filled with blood. Iron pigment granules were conspicuous within individual liver cells, as well as within Kupffer cells. The latter contained many phagocytosed erythrocytes. Bile thrombi plugged the lumina of some of the bile ducts. Occasional focal necroses were seen.

Spleen: The spleen showed red blood cell phagocytosis and deposition of iron-containing pigment. Neither the liver nor the spleen showed any evidence of extramedullary hemopoiesis.

Kidney: Many of the collecting tubules were greatly distended. Some tubular lumina were plugged by granular eosinophilic-staining debris, probably representing desquamated epithelium. The spaces of Bowman were occasionally distended and contained laminated calcium-containing bodies.

Brain: There was spotty absence of ganglion cells, especially in the third and fourth cortical layers, with very little glial reaction. A good many of the neurones which could be identified as such lacked Nissl substance and showed vacuolization of the cytoplasm and irregularity of cell membrane outlines.

Additional histologic findings included a thin adrenal cortex, with poor delimitation of the various zones and foci of bronchopneumonia, along with many septal cells and abundant edema fluid within pulmonary alveoli. The bronchial cartilages were normal. The other viscera showed no lesions of note.

COMMENT

In Case 1, intra- and extra-vascular aggregates of platelets in association with platelet thrombi, hemorrhages, hemosiderosis of the spleen, and an absolute increase of megakaryocytes in the bone marrow are features which prompt the diagnosis of thrombotic thrombocytopenic purpura. The fact that the megakaryocytes showed alterations in their structure suggests a response to the action of some toxic agent

in the fetal circulation. It is well known that many cases of secondary thrombocytopenic purpura are but a manifestation of an allergic, toxic, or infectious state.[37] During the course of the pregnancy the mother was treated for rheumatoid arthritis with gold and salicylates, which are capable of causing purpura. Gold is a toxic agent in its own right, and often its untoward effects are those of a response to sensitization. Secondary thrombocytopenic purpura due to salicylates is a far rarer phenomenon. Rappoport and co-workers, in 1945, reported the first such case.[38] The patient, a Negro soldier, had rheumatoid arthritis, for which large doses of sodium salicylate were prescribed. After a period of 33 days, therapy was discontinued, only to be reinstituted after a drug-free period of 15 days. Nine days later bleeding tendencies developed, which eventually proved fatal, despite splenectomy. It is interesting to note that bone marrow studies in Rappoport's case showed an abundance of megakaryocytes in which there were described degenerative changes similar to those in Case 1.

In Case 2 the clinical and pathologic data warrant no satisfactory diagnosis. The jaundice seems best explained as an obstructive phenomenon. The progressive clearing of the jaundice is difficult to explain, unless one regards it as evidence that the obstruction had been alleviated. The erythrophagocytosis is equally difficult to account for. Talbot notes that 40 to 50% of transfused blood, if it is two or more weeks old, is destroyed within the first few hours after administration.[39] The mechanism by which it is destroyed is not clear. The infant, in this instance nine weeks before death, had received 50 cc. of blood that was at least 20 days old. Even if erythrophagocytosis is one of the mechanisms by which old blood is removed, it does not seem likely that the process would go on for nine weeks with such striking activity. Barring this possibility, one is forced to view the erythrophagocytosis and the hemosiderosis of the spleen and liver as a manifestation of some hemolytic process superimposed on and contributing in some measure to obstructive jaundice. A negative Coombs test would rule out at least the factors of auto- and iso-hemagglutination in erythroblastosis. The lack of extramedullary hemopoiesis would also argue against such a diagnosis. Normal red blood cell fragility studies exclude congenital hemolytic anemia.

The cause for the renal tubular alteration seems most likely to be some obstructive process in the more distal segments of the collecting tubules. The kidney appears to play no major role in the total picture.

In comparing these two cases, it becomes evident that they represent two totally different generalized pathologic processes, both of which have affected cartilage. Thus, despite the fact that deposition of calcium salts in the substance of epiphyseal and other preosseous cartilages is common to the two, the histologic features of the two cases are quite dissimilar. Furthermore, after these microscopic differences have been seen and appreciated, there can be discerned in retrospect differences in the x-ray studies of the two infants. In the first case, the densities are characterized by being uniformly tiny and discrete, shot-like, and finely grained. They affect the preosseous cartilages of the axial, as well as appendicular, skeleton. In the second case there is a tendency toward coalescence, and the densities appear large and coarse and replace much of the cartilage. Only the cartilages of the appendicular skeleton are affected. Histologic examination suggests differing mechanisms for the cartilage abnormality in the two cases. In Case 1 calcium salts appear to have been deposited

in small foci at sites where there may have been previous hemorrhages or minute infarcts secondary to occlusion of the small vessels in the cartilage canals. Osteogenesis is generally unaffected. In Case 2 the ground substance is selectively disturbed, leading to massive necrosis of cartilage and subsequent organization. In the latter phase, calcium deposits make their appearance. Endochondral ossification in this instance is thwarted, because chemically altered ground substance at the actively growing zone does not undergo calcification, and the sequence of proliferation and maturation of cartilage cells, for all practical purposes, is nonexistent.

Lens and cartilage present certain striking similarities. They have a high content of mucopolysaccharide. In the lens it is hyaluronic acid, and in the hyaline cartilage it is chondroitin sulfate. Both lens and cartilage lack an intrinsic blood supply and carry on their metabolic activities by a process of diffusion. In this connection the mucopolysaccharides are thought to play an important rôle. In addition, it is thought that the refractive media of the eye, notably lens, cornea, and vitreous humor, owe their transparent properties in large part to hyaluronic acid.[40] Vascular channels are present in the larger cartilages, but there are no anastomoses between them. This provision is apparently an adaptive phenomenon. In man the smaller cartilages of the trachea and bronchi, for example, are devoid of any such vascular channels; whereas in large animals, such as the ox, these cartilages are well supplied with vessels [41]; on the other hand, the epiphyseal cartilages of the rat are devoid of cartilage canals. During development, beginning in the sixth week and continuing for a period of approximately 10 weeks, the lens has a blood supply derived from the hyaloid and iris vessels. In any disease in which physiologic processes common to lens and cartilage are affected, it is not unreasonable to expect manifestations of the disease to appear in both structures. These considerations may well serve as starting points toward elucidating the pathogenesis of this complex array of calcareous chondropathies.

SUMMARY

Forty-one reports of a calcareous type of chondropathy in newborn infants are reviewed. In addition, the clinical and pathologic details of two new cases are recorded.

Calcific deposits occur within the substance of preosseous hyaline cartilage, which in the roentgenogram presents a stippled appearance. The resultant deformities of the axial and/or appendicular skeleton are usually noted at birth. Bilateral congenital cataract and/or some type of dermatosis are associated with the skeletal disorder in approximately 25% of all cases.

The chondropathy is oftenest referred to as "chondrodystrophia calcificans congenita." This term and others like it place emphasis on the lesion of cartilage and tend to direct attention away from the possibility that the disorder may be secondary to a fundamental disease process affecting the entire organism. Illustrating this are the two new cases recorded here. In comparing them it becomes apparent that two totally different pathologic processes have effected somewhat similar changes in cartilage. Furthermore, differences in the x-ray studies of the two infants are paralleled by differences in the histologic features of the diseased cartilages. The stillborn infant in Case 1 had disseminated platelet thromboses; the 6-week-old infant in Case 2 had bilateral congenital cataract, hemolytic anemia, and an obscure disturbance of liver function marked by jaundice that cleared terminally. Liver func-

tion studies pointed to an obstructive jaundice. Histologic examination of autopsy material revealed bile thrombi in the liver and active erythrophagocytosis in and hemosiderosis of spleen and liver.

The pathogenesis of the chondropathies in these two cases is considered. The biochemical and physiologic similarities of lens and cartilage are compared. It is suggested that concurrent disease in the two structures may point to abnormalities in tissue mucopolysaccharide.

The calcareous chondropathies are not only the concern of the pediatrician, the roentgenologist, and the pathologist but also of the orthopedist, who may first see the patient because of skeletal defects, and of the ophthalmologist, who may first see him because of cataracts.

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Case Reports

FOCAL NECROTIZING GLOMERULONEPHRITIS AND DIFFUSE HYPERSENSITIVITY ANGIITIS

Report of Case with Documentation by Renal Biopsy Three Years Before Death

MAVIS A. KAUFMAN, M.D.
NEW YORK

THE ASSOCIATION of a particular type of focal glomerulonephritis with subacute bacterial endocarditis is well known.[1] In past years it has been referred to as "focal embolic glomerulonephritis," but more recently evidence has been presented to indicate that these lesions are not embolic in origin but represent fibrinoid necrosis of glomerular capillaries as a result of an altered immunoallergic reaction.[2] It is also apparent that, while these lesions occur predominantly in association with subacute bacterial endocarditis, they may also be observed in instances of diffuse allergic angiitis or as part of a hypersensitivity reaction to various drugs.[2] Accordingly, the term "focal necrotizing glomerulonephritis" has recently been suggested,[2] instead of the pathogenetically incorrect "focal embolic glomerulonephritis" or the restricted term "focal endocarditic glomerulonephritis." The case to be presented illustrates the lesion in the absence of endocarditis. Furthermore, it affords an unusual opportunity for comparison of renal specimen taken for biopsy with autopsy material obtained three years later.

REPORT OF CASE

A 33-year-old white Puerto Rican man was admitted to the hospital for the fifth time on Feb. 6, 1953, complaining of severe shortness of breath. Past history revealed frequent headaches and a diagnosis of hypertension in 1949. In August, 1950, he was hospitalized for the first time because of substernal oppression and dyspnea. The blood pressure was 180/150. The heart was enlarged, and serial electrocardiograms showed left ventricular strain but no infarction. The urine specific gravity was 1.014, with 4+ albumin, three to five white blood cells per high-power field, and several fine and coarsely granular casts. On one occasion a few red blood cells were seen per high-power field. Renal function studies and blood urea nitrogen were normal. Retrograde pyelography showed an extrinsic mass compressing and displacing the upper calyces of the left kidney posteriorly. In view of this and other evidence suggesting an adrenal tumor, bilateral adrenal exploration was done. The spleen was found to be three to four times its normal size. The adrenal glands were normal in size and configuration. Specimens for biopsy were taken from the spleen and left kidney (Fig. 1). Postoperatively he received 100,000 units of penicillin every six hours for 14 days. He was digitalized and discharged symptom-free.

Six months later, in August, 1951, he was again admitted because of paroxysmal nocturnal dyspnea, occasional precordial constrictive pain, and blood-tinged urine. He was found to be in mild congestive failure, but again serial electrocardiograms showed no infarction. The blood pressure was 182/148. The urine specific gravity was 1.012, with 4+ albumin, 10 to 15 red blood cells and 4 to 6 white blood cells per high-power field, and 0 to 1 granular cast. Gross hematuria was not noted. During hospitalization routine examination revealed asymptomatic reddening of the pharynx. He was given intramuscular penicillin, 300,000 units twice a day for three days. Five days after the cessation of therapy an urticarial skin eruption appeared. This was considered a manifestation of penicillin sensitivity and disappeared after therapy with antihistamine drugs. He was discharged after redigitalization.

From the Laboratory Service, Veterans Administration Hospital, Bronx, N. Y.

He was seen again in January, 1952, because of identical symptoms and mild congestive failure, which abated with redigitalization. The blood pressure remained elevated. Renal function studies showed increasing impairment, but the blood urea nitrogen was normal. The urinalysis was similar to that on the previous admission but showed an increase in the red blood cells to between 20 and 25 per high-power field.

He returned in September, 1952, with similar complaints and again was found to be in mild congestive failure. Although physical signs and symptoms on the third hospital day suggested a pulmonary infarction, roentgenograms failed to substantiate the diagnosis. No source for emboli was found. He was given penicillin, 600,000 units daily for 16 days, and no sensitivity reaction was observed.

The last hospitalization was in February, 1953, when he was transferred from another hospital to which he had been admitted in January. He was dyspneic and slightly cyanotic and com-

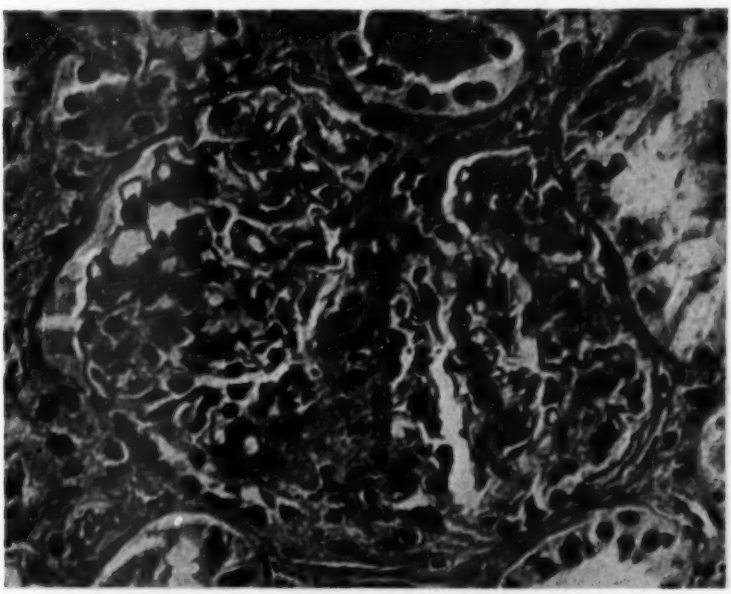

Fig. 1.—Focal necrotizing glomerulonephritis. Section from biopsy. Hematoxylin and eosin; × 475.

plained of right lateral chest pain. The blood pressure was 170/120. The liver was enlarged and tender, and there was an abdominal fluid wave. Chest roentgenogram revealed an infarct at the base of the right lung. The blood urea nitrogen was normal on admission. The urine specific gravity was 1.022. There was 4+ albuminuria, with occasional red blood cells and white blood cells per high-power field. Serum cholesterol was 174 mg. per 100 cc., and cholesterol esters were 117 mg. per 100 cc. Total serum protein 4.8 gm. per 100 cc., with the albumin 3.2 gm. and the globulin 1.6 gm. per 100 cc. Anemia was present for the first time. The red blood cell count was 3,240,000 per cubic millimeter, and the hemoglobin was 10.8 gm. The temperature on admission was 100 F.

He again received penicillin, 300,000 units twice a day for three days, followed by 100,000 units every three hours until death four days later, without any demonstrable reaction. Therapy was first directed toward alleviation of what appeared to be predominantly right-sided heart failure. Eventually, it was concerned chiefly with maintaining electrolyte balance as the blood urea nitrogen began to rise. At the same time the urinary output fell, and urine analyses showed

4+ albumin, 50 white blood cells, and over 100 red blood cells per high-power field. The terminal blood chemistry values were urea nitrogen 115 mg. per 100 cc., CO_2 45 volumes per 100 cc., sodium 134 mEq. per liter, potassium 6.8 mEq. per liter, serum chlorides 96 mEq. per liter. The white blood cell count rose to 15,200, with 87% polymorphonuclear leucocytes, of which 6% were band forms, 5% lymphocytes, 7% monocytes, and 1% eosinophiles. No eosinophilia had ever been present, and all previous blood cell counts had been normal. He died in uremia in March, 1953, approximately one month after admission.

Autopsy Findings.—An autopsy was performed three hours after death. The skin of the abdomen and back showed scattered small purpuric areas. There were a few petechiae over the chest. Each pleural cavity contained about 500 cc. of serous fluid, and the abdominal cavity contained a large quantity of ascitic fluid. The heart weighed 680 gm. and showed marked hypertrophy, predominantly of the left ventricle. The apex of the left ventricular cavity was

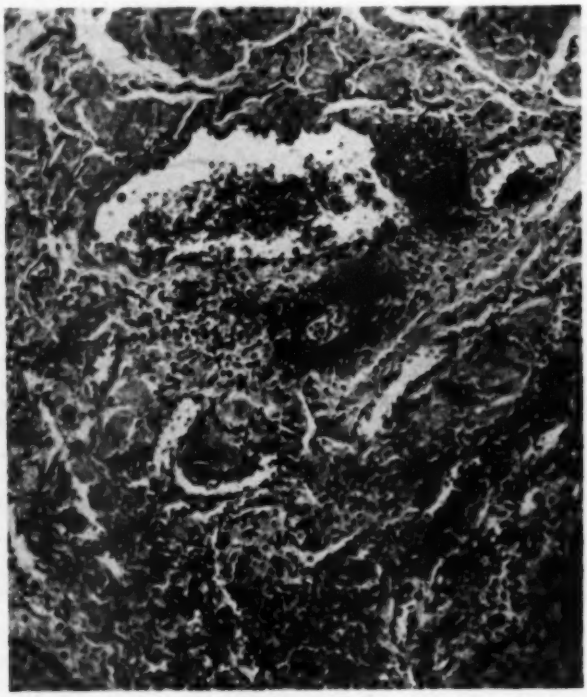

Fig. 2.—Esophagus showing fulminant fibrinoid necrosis of arterioles and diffuse polymorphous infiltration. Focal necrosis of venule walls by contiguity. Hematoxylin and eosin; × 210.

filled with friable mural thrombus, and there was a small pedunculated mass of similar material over the midportion of its septal surface. The underlying myocardium and the valves were normal. There was minimal sclerosis of the anterior descending ramus of the left coronary artery, although all the vessels appeared somewhat small in relation to the size of the heart. A blood culture taken from the left ventricle was sterile. The lungs contained numerous fresh and slightly older infarcts varying in greatest dimension from 2 to 5 cm. The smaller infarcts were occasionally located in the central portions of the lungs. There were many petechiae of the parietal and visceral pleurae. The spleen was moderately enlarged and weighed 500 gm. There were numerous small fresh infarcts in the central portions of the spleen and an old infarct immediately beneath the capsule. The hilar vessels, like those of the lungs, were fully patent. The gastrointestinal tract showed multiple minute submucosal hemorrhages, but there was no

blood in the lumen. The kidneys weighed 180 and 190 gm. They were of average size, smooth-surfaced, and soft. When sectioned, the cortices bulged above the capsules and were yellowish tan, with a patchy loss of striations. The medullae, in sharp contrast to the pale cortices, were a uniform deep red. The flaring medullary vessels were still discernible. The pelves and ureters were lined by thickened velvety deep red mucosa. The right ureter was filled with a fresh blood clot, and the lumen of the left ureter was greatly narrowed by the swollen hemorrhagic mucosa. The bladder mucosa was also mottled with hemorrhages.

Microscopic Examination.—There was a diffuse vascular lesion of striking uniformity involving small arteries, arterioles, and occasionally venules in the gastrointestinal and genitourinary tracts, gall bladder, spleen, pancreas, lungs, and skin (Figs. 2, 3, and 4). It was characterized by an intense fibrinoid necrosis and swelling of the entire vessel wall, often resulting in a marked narrowing of the lumen.

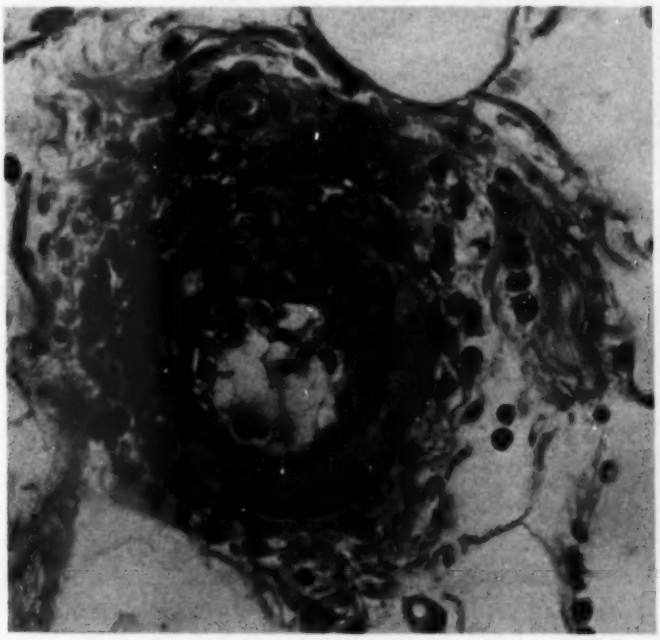

Fig. 3.—Small artery in submucosa of small intestine showing focal disruption of elastica, fibrinoid necrosis, and polymorphonuclear leucocyte infiltration. Hematoxylin and eosin; × 627.

There was a diffuse polymorphonuclear infiltration of the wall and the surrounding tissues. Eosinophiles were rare. It was frequently impossible to determine whether one was dealing with a small artery or vein, so complete was the destruction. In segments of ureter, renal pelvis, and gastrointestinal tract all small vessels were uniformly affected. Rupture of the damaged walls and widespread hemorrhages were frequent. Less severely damaged small arteries showed fibrinoid necrosis of the adventitia and outer media. Polymorphonuclear leucocytes infiltrated the entire wall, including the slightly swollen intima. Occasionally only a portion of the circumference was involved. Rarely, as in one small pulmonary artery, the involved portion was at a bifurcation. Thrombosis was frequent, especially of the small pulmonary arteries,

and resulted in multiple areas of infarction. Many other small arteries and arterioles showed only an intense fibrinoid necrosis, and the inflammatory reaction was less prominent in the wall, although the surrounding tissues were infiltrated.

Although many of the splenic infarcts were on the same basis as those in the lung, one small artery contained material so similar to that of the mural thrombus in the heart that a double mechanism was apparently operative there (embolization from the heart and thrombosis secondary to the focal arteritis).

The underlying myocardium revealed no clue as to the origin of the mural thrombus. However, since the thrombus was sufficiently old to show organization at its myocardial attachment, one may postulate some earlier disturbance of the endocar-

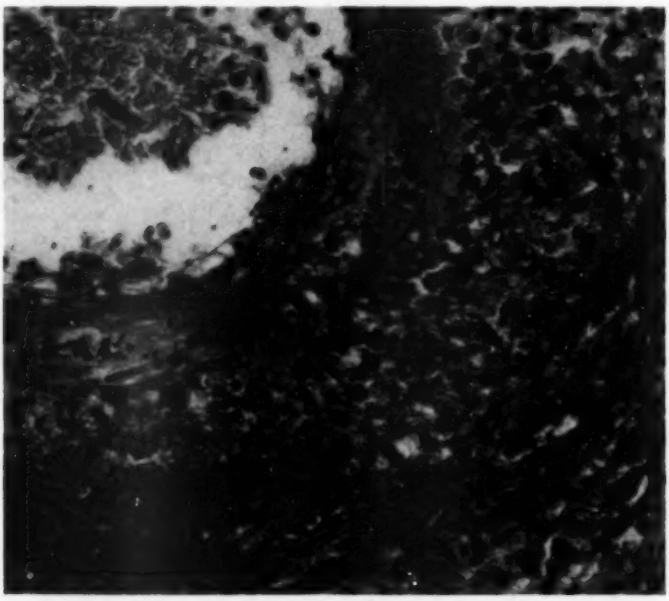

Fig. 4.—Periureteral artery showing fibrinoid necrosis of wall, complete disruption of elastica, and polymorphonuclear leucocyte infiltration in and around vessel wall. Hematoxylin and eosin; × 475.

dium, perhaps vascular lesions similar to those elsewhere, traces of which were effaced by coalescence with the mural thrombus.

The small arteries of the skin in sections through the purpuric areas showed marked polymorphonuclear perivascular infiltration without actual necrosis.

Vascular lesions were found in the kidneys, renal pelves, ureters, bladder, prostate, and testes. However, the majority were in the ureters and pelves, where almost all the small vessels showed fulminant fibrinoid necrosis and rupture of their walls. The arterioles of the medulla had undergone similar disruption, resulting in diffuse interstitial hemorrhages. The arteriolae verae rectae were mainly involved, and only a few arterioles at the corticomedullary boundary were affected. The larger renal

arteries at the hilus, the interlobar and arcuate arteries, were normal. The arterioles of the cortex, in striking contrast to those in the medulla, were almost all normal. The main vascular changes were confined to portions of the glomerular capillaries.

The glomerular lesion was identical to that found in the specimen taken for biopsy three years earlier (Figs. 1 and 5). The earliest changes appeared to consist of a swelling of both the endothelium and the epithelium in one or two segments of the capillary tuft. Adhesions between the damaged loops and Bowman's capsule, with proliferation of epithelial crescents, were frequent. Further changes consisted of a fibrinoid necrosis of the involved loops and fusion into a hyalinized mass, retaining the

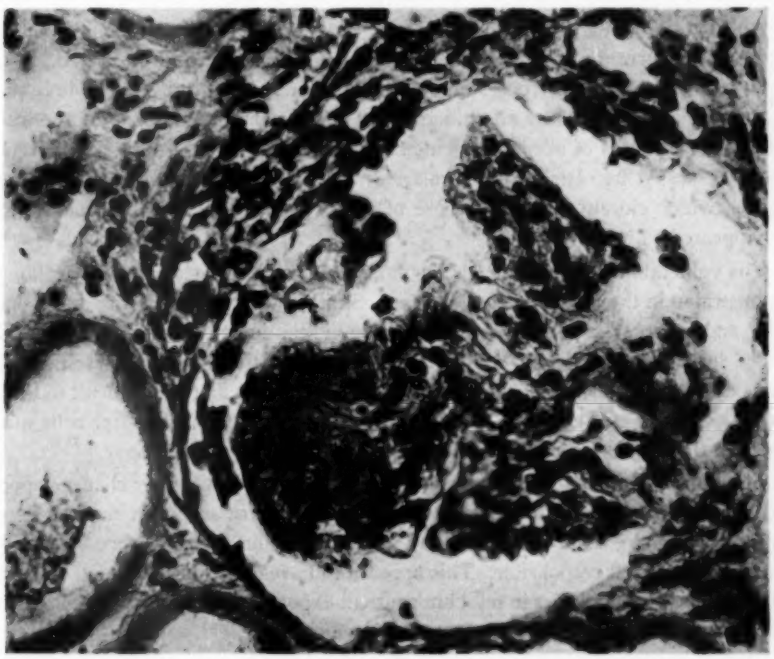

Fig. 5.—Focal necrotizing glomerulonephritis. Section from autopsy material. Hematoxylin and eosin; × 475.

original configuration. The adjacent portions of the glomeruli were normal. Interstitial lymphocytic infiltration was inconstant but occasionally surrounded a damaged glomerulus. No arterial or arteriolar lesions were found in the specimen taken for biopsy. The convoluted tubules contained red blood cells and traces of hemoglobin at autopsy. The postmortem specimen contained wide areas of interstitial fibrosis filled with atrophic tubules and scattered hyalinized glomeruli, indicating a previous healed lesion. Many spared glomeruli were present. However, a number of the glomerular lesions appeared more fulminant than those in the material for biopsy. These glomeruli were completely necrotic, infiltrated by polymorphonuclear leucocytes, and surrounded by small hemorrhages and scattered polymorphonuclear leuco-

cytes. The virtual sparing of the afferent arterioles was of particular interest, in view of the four-year history of hypertension. None of the changes characteristic of malignant hypertension were found in the kidneys or other viscera. The essential autopsy diagnoses were focal necrotizing glomerulonephritis; necrotizing angiitis (probably hypersensitivity angiitis) of lungs, gastrointestinal and genitourinary tracts, gall bladder, spleen, and skin; infarcts of lungs and spleen (recent and fresh); moderate splenomegaly; hypertrophy of the heart, particularly of the left ventricle; slight coronary sclerosis; mural thrombi of left ventricle; bilateral pleural effusion; ascites; chronic passive congestion of lungs and liver; clinical hypertension.

COMMENT

An allergic etiology of focal necrotizing glomerulonephritis has long been suspected. Its occurrence in association with vascular lesions also of suspected allergic origin would seem to be strong support for this view. Allen [2] referred to four cases which were accompanied by allergic arteritis. Churg and Strauss [3] noted the lesion in two of their cases of allergic granulomatous arteritis. Zeek, Smith, and Weeter [4] briefly described necrotizing glomerular lesions in seven cases of hypersensitivity angiitis, which closely resembled those of the present case. The vascular lesions also appeared to be identical.

The vulnerability of the vascular system to a variety of substances has been amply demonstrated in the reports of Zeek [5]; Zeek, Smith, and Weeter [4]; More and Kobernick,[6] and others. In particular, necrotizing angiitis due to penicillin has been described by Berne,[7] Harkavy,[8] and Waugh.[9] It is tempting to incriminate penicillin in this case. However, the documentation is incomplete, in that it is difficult to fortify this impression with the lack of clinical evidence of allergic reaction after subsequent penicillin therapy and the absence of healed vascular lesions at autopsy.

The differences between the glomerular and generalized vascular changes appear to be quantitative and not qualitative. Therefore, one must suspect a similar allergic origin. Since these lesions preceded any known penicillin therapy, some other allergen must have been responsible. This appears to have acted initially upon segments of the glomerular capillaries in mild intermittent exposures. Finally, an overwhelming exposure resulted in the fulminant necrotizing angiitis.

SUMMARY

The patient was a 33-year-old white man with a history of four previous hospitalizations for dyspnea and chest pain. He was found to have hypertension, an enlarged heart, and albuminuria, with the subsequent appearance of microscopic hematuria. The last admission, three years after the first, was characterized by refractory heart failure, pulmonary infarction, and a late, rather sudden elevation of the blood urea nitrogen associated with gross hematuria and death. The primary autopsy diagnoses were necrotizing angiitis (probably hypersensitivity angiitis) and focal necrotizing glomerulonephritis. The relationship of the two conditions and their occurrence as manifestations of hypersensitivity are briefly discussed.

Guidance in the interpretation of the pathologic material and in the preparation of this report was given by Dr. Arthur C. Allen.

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News and Comment

ANNOUNCEMENTS

Seventh International Cancer Congress.—Physicians and scientists are invited to present papers at the Seventh International Cancer Congress, to be held in São Paulo, Brazil, July 23-29, 1954, under the sponsorship of the International Union Against Cancer. The program will include sections on fundamental cancer research, on clinical studies on cancer, and on cancer control. Registration blanks are available from the Chairman, National Committee on the International Union Against Cancer, National Research Council, 2101 Constitution Avenue, N. W., Washington 25, D. C.

It is expected that round trip transportation by air from Miami to São Paulo will be available for about $480. Detailed information regarding travel arrangements and hotel reservations may be obtained from Dr. Brewster S. Miller, American Cancer Society, Inc., 47 Beaver Street, New York 4.

In accordance with similar arrangements being made in other countries to coordinate participation in the Congress, residents of the United States who desire to present papers must send five copies of an abstract of each paper proposed for presentation to the Chairman, National Committee on the International Union Against Cancer, at the above address by Jan. 15, 1954. Abstracts are not to exceed 250 words and must be accompanied by a title and the name, address, academic or professional title, and institutional affiliation of the investigator or physician. These requirements do not apply to people who have been invited to participate by the President of the Congress, unless application is made for travel allotments as described below.

Travel allotments of approximately $600 each will be available to a limited number of persons requiring such assistance. Applications for travel allotments must be submitted in quintuplet to the Chairman, National Committee on the International Union Against Cancer, at the above address by Jan. 15, 1954. They should be in letter form giving information concerning age, training, publications in cancer or related fields, and academic or professional status. Applicants not planning to present papers should include five copies of abstracts, as described above, of major current investigative work. A letter from the laboratory director, or appropriate administrative officer, approving the application is also necessary.

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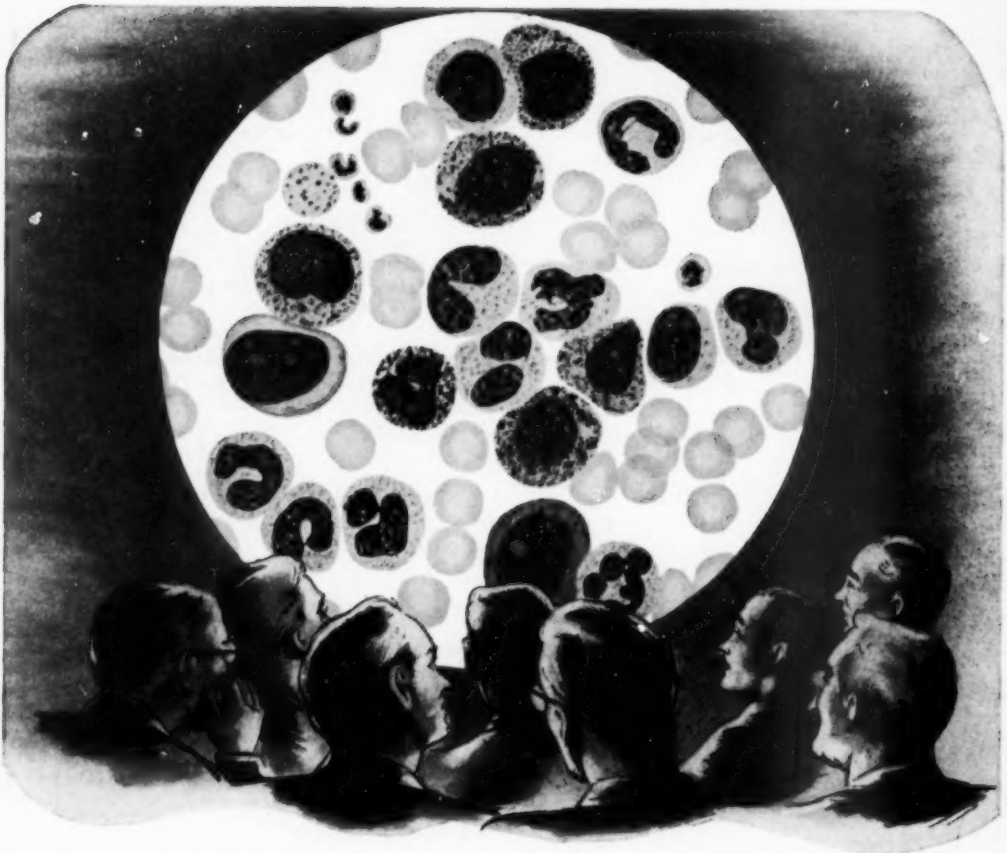

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